chapter

20 Molecular Genetics

In this chapter

- Exploration: Similarities and Differences
- Mini Investigation: Building a DNA Model
- Web Activity: DNA Replication
- Lab Exercise 20.A: Synthesis of a Protein
- Investigation 20.1: Protein Synthesis and Inactivation of Antibiotics
- Web Activity: Electrophoresis
- Web Activity: Researchers in Human Genetic Disorders
- Mini Investigation: Examining the Human Genome
- Investigation 20.2: Restriction Enzyme Digestion of Bacteriophage DNA
- Web Activity: Transformation of Eukaryotes
- Case Study: Gene Mutations and Cancer
- Lab Exercise 20.B: Looking for SINEs of Evolution

By the mid-1950s, scientists had determined that chromosomes contained DNA and that DNA was the genetic material (**Figure 1**). Building on the work of other scientists, Watson and Crick deduced the structure of this complex molecule. This knowledge laid the basis for the field of molecular biology, which aims to understand the inheritance of traits at the level of interactions between molecules in the cell.

A primary goal of molecular genetics is to understand how DNA determines the phenotype of an organism. What happens to DNA during duplication of chromosomes in mitosis? How does the structure of DNA relate to its function? How does one molecule, identical in every somatic cell of an organism, determine the characteristics of the many different types of cells that are found in that organism?

Today, questions such as these continue to drive research in the fields of biology, biotechnology, biochemistry, and medicine. We now know the sequence of all the nucleotides that make up the genome of many organisms, including that of our own species, *Homo sapiens*. This information has given scientists new ways to study the relationships between species and the mechanisms of evolution. It also allows law enforcement agencies to identify individuals with incredible accuracy from minute quantities of DNA.

Using genetic technologies, scientists can move genes from one species to another. In fields such as agriculture, corporations have patented the genomes of these organisms in order to profit from the advantages they offer over conventional organisms. Similar manipulation of human cells may one day lead to treatments for previously untreatable debilitating diseases. The research and application of these technologies raises many social, ethical, and legal issues that society has yet to fully resolve.

STARTING Points

Answer these questions as best you can with your current knowledge. Then, using the concepts and skills you have learned, you will revise your answers at the end of the chapter.

1. Differentiate between DNA and proteins. What cellular roles do they play?
2. Describe the physical and chemical characteristics of DNA.
3. What is the significance of DNA replication in your body?
4. Write a short overview, in paragraph form, of the process of DNA replication.

Career Connections:
Biological Technician; Biotechnologist

Figure 1
DNA sequences are represented by the letters A, T, C, and G.

Exploration — *The Size of the Genome*

All organisms, no matter how simple they may seem to us, require DNA in each cell to encode the instructions necessary to live and reproduce. The total DNA of an organism is referred to as its genome. In bacteria, the genomic DNA is circular, accounts for 2 % to 3 % of the cell's mass, and occupies about 10 % of its volume. In this activity, you will make a model of an *Escherichia coli* cell that will be 10 000 times bigger than actual size. You will also gain an appreciation for how compactly DNA is packed within a cell.

Materials: 2 cm gelatin capsule, 10 m of white thread, 10 m of coloured thread

- Try to construct the bacterium by placing the long lengths of thread inside the gelatin capsule. Good luck! It's not easy!

(a) Why does it take two lengths of thread to represent the chromosome?

(b) Is the thread that you tried to place in the capsule too thick to represent the actual thickness of the DNA? (What percentage of bacterial cell volume does your thread fill, and what is the actual volume that the DNA occupies in the bacteria?)

(c) If the human genome is 1000 times bigger than the *E. coli* genome, how many metres of thread would it take to represent the human genome?

(d) What size container would you need to hold the thread representing the human genome?

20.1 DNA Structure and Replication

According to the model proposed by Watson and Crick, DNA consists of two strands of nucleotides. Each nucleotide contains a deoxyribose sugar, a phosphate group, and a nitrogenous base, all covalently bonded to each other. Each strand of DNA has a backbone of sugar and phosphate groups (**Figure 1**). The nitrogenous bases stick out from the backbone of each DNA strand.

Watson and Crick's model also indicates that the two strands of DNA form a structure that resembles a twisted ladder. The base pairs are the rungs of the ladder and the sugar–phosphate backbones are the struts. This structure is called a double helix (see **Figure 1**). Each DNA strand in the double helix twists in a clockwise direction.

CAREER CONNECTION

Biological Technician

Biological technicians may work in the field, in the laboratory, or both. They perform routine analysis and technical duties to support the work of scientists and engineers working in fields that include molecular biology. What educational background is required to enter this field?

www.science.nelson.com GO

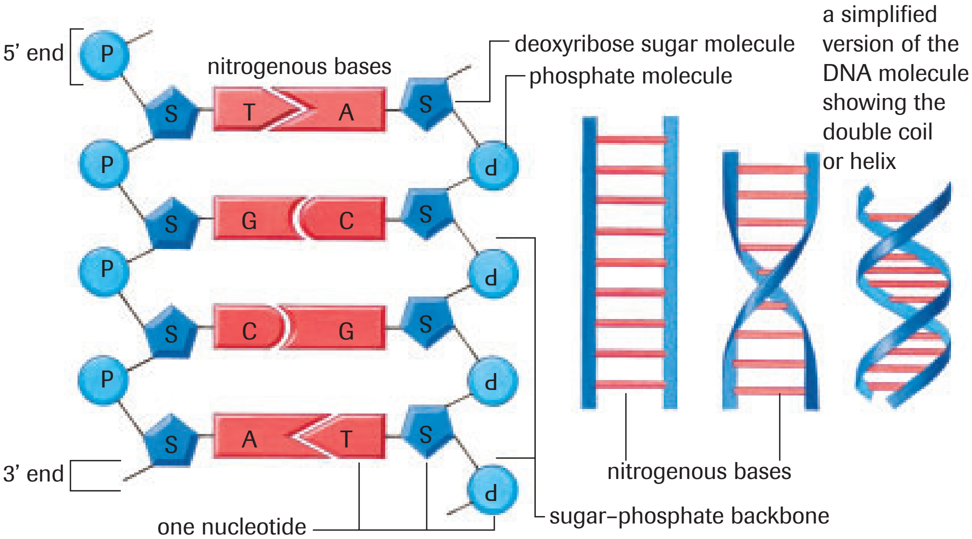


Figure 1
A simplified diagram of the structure of DNA

In the DNA molecule, the bases of one DNA strand are paired with bases in the other strand. A purine is always paired with a pyrimidine. Adenine (a purine) is always paired with thymine (a pyrimidine), and guanine (a purine) is always paired with cytosine (a pyrimidine). This type of pairing is termed **complementary base pairing**. Hydrogen bonds, between the complementary bases (A-T and G-C) on opposite strands, hold the double helix together (**Figure 2**). Although a single hydrogen bond is very weak, large numbers of hydrogen bonds are collectively strong, so the DNA molecule is very stable.

complementary base pairing pairing of the nitrogenous base of one strand of DNA with the nitrogenous base of another strand

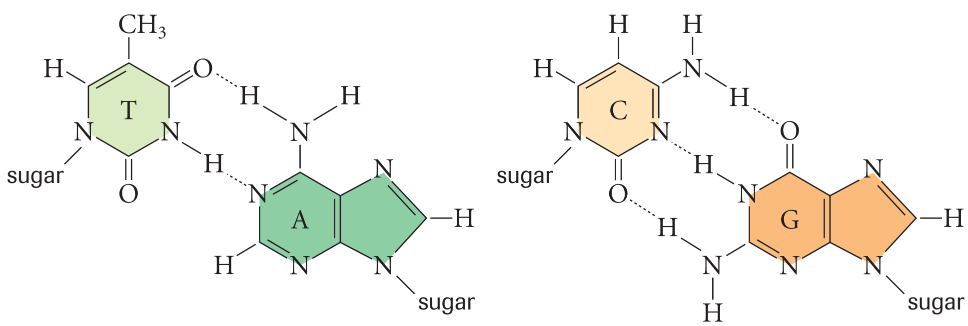


Figure 2
Adenine forms two hydrogen bonds with thymine, while guanine forms three hydrogen bonds with cytosine.

The sequence of bases on any one strand of DNA can vary greatly between species, but its opposite strand will always have the complementary sequence of bases. For example, the sequences of the strands below are complementary:

5′–ATGCCGTTA–3′
3′–TACGGCAAT–5′

The two strands of nucleotides are **antiparallel**. They run parallel but in opposite directions to one another. One strand will have a 5′ carbon and phosphate group at one end and a 3′ carbon and the hydroxyl group of a deoxyribose sugar at its other end. Its antiparallel strand will have a 3′ carbon and the hydroxyl group of a deoxyribose sugar at the first end and a 5′ carbon and phosphate group at its other end (**Figure 1**, previous page).

The direction of the strand is important when enzymes interact with DNA, either to copy the DNA prior to cell division or to "read" genes in order to make proteins. Enzymes can read or copy DNA in only one direction. The sequence of only one DNA strand is given when sequences are written out since the complementary strand is easily deduced according to the rules of complementary base pairing.

antiparallel parallel but running in opposite directions; the 5′ end of one strand of DNA aligns with the 3′ end of the other strand in a double helix

Learning Tip

The rules of complementary DNA base pairing are
- A to T
- G to C

When you know the sequence on one strand, you also know the sequence on the complementary strand.

Practice

1. Define the following terms: nucleotide, complementary base pairing, and antiparallel.
2. In a DNA molecule, a purine pairs with a pyrimidine. If this is the case, then why can't A–C and G–T pairs form? (*Hint:* Look closely at the bonds between the base pairs in **Figure 2** on the previous page.)
3. The following is a segment taken from a strand of DNA: 5′–ATGCCTTA–3′. Write out the complementary strand for this segment. Be sure to show directionality.

mini *Investigation* Building a DNA Model

What would a section of a DNA molecule look like if you could see one close up? You can find out by building your own model of the double helix. For this activity, you need to select materials that will allow you to model the following features:

- the sugar–phosphate backbone
- the anti-parallel strands
- the four different nitrogenous bases
- the bonds between complementary base pairs that hold the two strands together

Your model should show a minimum of 12 base pairs. It should be free-standing and approximately 15 cm tall by 6 cm wide. Include a legend with your model that clearly identifies each part of the DNA strand.

DNA Replication

In Chapter 17, you saw that mitosis involves the duplication of chromosomes. For mitosis to occur, DNA must copy itself and be equally divided between the daughter cells. To have all the correct genetic information, the DNA in each daughter cell must be an exact copy of the DNA in the parent cell. **DNA replication** is the process by which a cell makes an exact copy of its DNA. The main stages of DNA replication are the same in both prokaryotic cells (without a membrane-bound nucleus) and eukaryotic cells (with a membrane-bound nucleus).

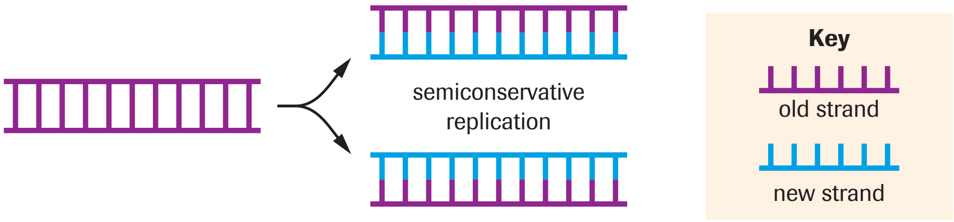
DNA replication is semiconservative. **Semiconservative replication** involves separating the two parent strands and using them to synthesize two new strands (**Figure 3**, next page). The hydrogen bonds between complementary bases break, allowing the DNA helix to unzip. Each single DNA strand acts as a **template** to build the complementary strand. Finally, any errors are repaired, resulting in two identical DNA molecules, one for each daughter cell.

DNA replication the process whereby DNA makes exact copies of itself

semiconservative replication process of replication in which each DNA molecule is composed of one parent strand and one newly synthesized strand

template a single-stranded DNA sequence that acts as the guiding pattern for producing a complementary DNA strand

Figure 3
DNA replicates semiconservatively. Each daughter molecule receives one strand from the parent molecule plus one newly synthesized strand.



Separating the DNA Strands

DNA helicase the enzyme that unwinds double-helical DNA by disrupting hydrogen bonds

The two strands of the DNA helix cannot simply pull apart because they are tightly held together by the hydrogen bonds between bases and by the twists of the helix. The enzyme **DNA helicase** unwinds the helix by breaking the hydrogen bonds between the complementary bases. As this happens, the bonds between bases tend to reform. To prevent this, proteins bind to the separated DNA strands, helping to hold them apart. The two strands are now separated along part of the DNA molecule and are the template strands for the next step in replication. The point at which the two template strands are separating is called the replication fork. One template strand runs in the 3′ to 5′ direction in relation to the replication fork, while the other runs in the 5′ to 3′ direction (**Figure 4**).

DID YOU KNOW?

DNA Polymerases

There are several DNA polymerases in a cell, all with their own role. Each has a unique name, created by adding a roman numeral after "DNA polymerase." The main DNA polymerase involved in DNA replication is DNA polymerase III. It adds the 5′ phosphate group of a free nucleotide to the 3′ carbon of the sugar in the last nucleotide.

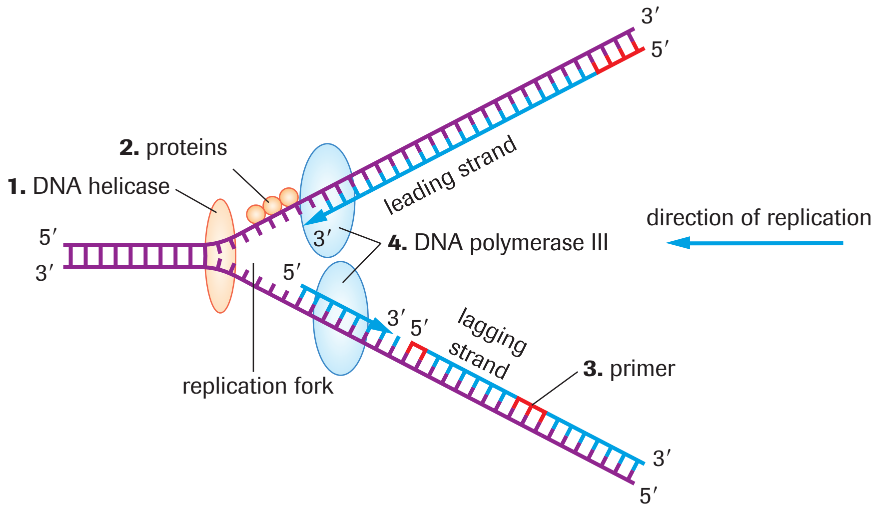


Figure 4
1. DNA helicase opens the double helix. 2. Proteins bind to the DNA to keep the two strands separate. 3. RNA primers are attached to the template strands. 4. DNA polymerase synthesizes the new DNA strands. The leading strand is synthesized continuously, and the lagging strand is synthesized in short fragments. DNA polymerase III adds complementary nucleotides in the 5′ to 3′ direction, using single-stranded primers as starting points. One nucleotide is attached to the next by bonding the phosphate on the 5′ end of the new nucleotide to the hydroxyl group on the 3′ end of the last nucleotide.

Building the Complementary Strands

DNA polymerase III the enzyme that synthesizes complementary strands of DNA during DNA replication

The next stage of DNA replication synthesizes two new DNA strands on the template strands through complementary base pairing. The new strands are synthesized by an enzyme called **DNA polymerase III**. This DNA polymerase builds a new strand by linking together free nucleotides that have bases complementary to the bases in the template. A short piece of single-stranded ribonucleic acid, called a primer, is attached to the template strand. This gives DNA polymerase III a starting point to begin synthesizing the

new DNA strand. DNA polymerase III adds nucleotides to a growing strand in *only one direction*—the 5′ to 3′ direction. The phosphate group at the 5′ end of a free nucleotide is connected to the hydroxyl group on the 3′ carbon of the sugar on the last nucleotide in the strand. As a result, one of the new strands will be synthesized continuously as DNA polymerase III moves in the 5′ to 3′ direction toward the replication fork. This strand is called the **leading strand**.

The other new strand, the **lagging strand**, is synthesized in short fragments. This allows the lagging strand to be synthesized in the 5′ to 3′ direction. RNA primers are required. To complete the replication of the DNA, the primers are cut out from the lagging strand and are replaced by DNA nucleotides by a different enzyme called **DNA polymerase I**.

Another enzyme, **DNA ligase**, links the sugar–phosphate backbone of the DNA fragments together (**Figure 5**).

leading strand the new strand of DNA that is synthesized towards the replication fork and continuously during DNA replication

lagging strand the new strand of DNA that is synthesized away from the replication fork and in short fragments, which are later joined together

DNA polymerase I an enzyme that removes RNA primers and replaces them with the appropriate nucleotides during DNA replication

DNA ligase an enzyme that joins DNA fragments together

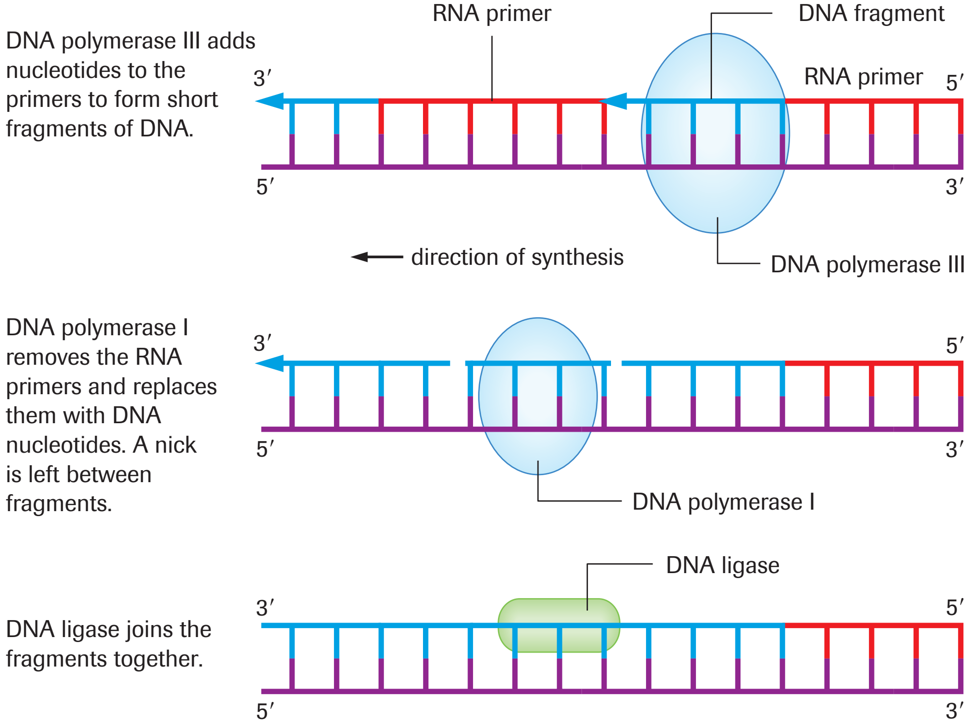


Figure 5
Building the lagging strand

DNA Repair

As complementary strands of DNA are synthesized, both DNA polymerase I and III act as quality control checkers by proofreading the newly synthesized strands. When a mistake occurs, the DNA polymerases backtrack to the incorrect nucleotide, cut it out, and then continue adding nucleotides to the complementary strand. The repair must be made immediately to avoid the mistake from being copied in later replications. Other DNA repair mechanisms can correct any errors that were missed during proofreading.

DID YOU KNOW?

Okazaki Fragments

The short fragments that are synthesized to form the lagging strand during DNA replication are called Okazaki fragments. They were named after Reiji Okazaki, who first described them in the 1960s.

WEB Activity

Simulation—DNA Replication

The *Escherichia coli* genome consists of 4.7 million nucleotide pairs. This entire genome is replicated in 40 min. Proofreading by DNA polymerase I and polymerase III maintains the error rate at roughly one error per 1000 cells duplicated! View a complete animation of DNA replication by accessing the Nelson Web site.

www.science.nelson.com GO

SUMMARY DNA Structure and Replication

Separating the Strands

- DNA helicase unzips the double helix by breaking the hydrogen bonds between the complementary bases in the two strands of the parent DNA molecule.
- Proteins attach to the newly exposed DNA strands, preventing the hydrogen bonds from re-forming and keeping the strands apart.

Building the Complementary Strands

- DNA polymerase III adds complementary nucleotides to the growing strands, using the exposed strands of the parent DNA molecule as a template.
- The leading strand is formed continuously.
- The lagging strand is formed in short fragments, starting from an RNA primer.
- DNA polymerase I cuts out the RNA primers and replaces them with the appropriate DNA nucleotides.
- DNA ligase joins the fragments together to form a complete DNA strand.

DNA Repair

- DNA polymerase enzymes cut out incorrectly paired nucleotides and add the correct nucleotides in a process called proofreading.

Section 20.1 Questions

1. Summarize the key physical and chemical properties of DNA.
2. Differentiate between a purine and a pyrimidine.
3. Copy **Table 1** into your notebook, fill in the missing information, and supply an appropriate title.

Table 1

Enzyme	Function
DNA helicase	
DNA polymerase I	
DNA polymerase III	
DNA ligase	

4. A molecule of DNA was analyzed and found to contain 20 % thymine. Calculate the percentage of adenine, guanine, and cytosine in this molecule.
5. Define a replication fork.
6. In a double helix, there is a complete turn every 3.4 nm, or 10 nucleotides. Assume that the DNA molecule in a particular chromosome is 75 mm long. Calculate the number of nucleotide pairs in this molecule.
7. Copy **Table 2** into your notebook and complete the missing information. Explain how you determined the missing values.

Table 2

Nucleotide	Sample A	Sample B	Sample C
adenine	10 %		20 %
guanine	40 %	15 %	
thymine		35 %	20 %
cytosine			

Gene Expression 20.2

As you learned in previous chapters, specific segments of DNA on a chromosome are called genes. Genes determine the inherited characteristics, or traits, of an organism. Every somatic (body) cell in an organism contains identical copies of DNA, and each of these DNA copies is a genetic blueprint for the organism. Once scientists knew the structure of DNA and how it replicated, they used this knowledge to further investigate another question: How do the genes in DNA determine an inherited trait?

The way the information in a gene is converted into a specific characteristic or trait through the production of a polypeptide is called **gene expression**. Recall that a polypeptide is a chain of amino acids and that proteins are made up of polypeptides. Proteins form many structures in an organism, such as skin and muscle, and they also form all of the enzymes in a cell. *The products of all genes are polypeptides.*

gene expression conversion of a gene into a specific trait through the production of a particular polypeptide

A second type of nucleic acid is involved in converting the instructions in a gene into a polypeptide chain. **Ribonucleic acid** (**RNA**) is a polymer of nucleotides similar to DNA. There are three main structural differences between RNA and DNA. First, the sugar in RNA has an extra hydroxyl group and is called ribose rather than deoxyribose (**Figure 1**). Second, instead of the base thymine found in DNA, RNA contains the base uracil. Like thymine, uracil can form complementary base pairs with adenine (**Figure 2**). Third, RNA is single-stranded and not double-stranded like DNA. There are three types of RNA that are needed to convert genes into proteins: messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA).

ribonucleic acid (RNA) a nucleic acid consisting of nucleotides comprised of the sugar ribose and nitrogenous bases

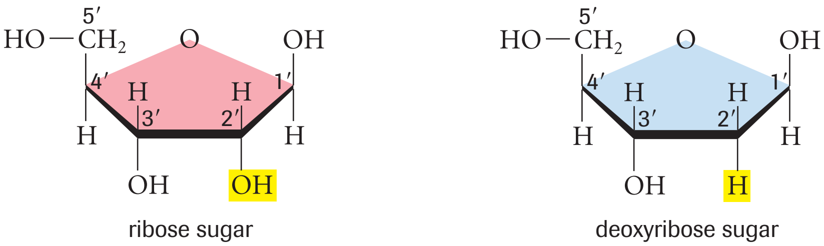


Figure 1
A ribose sugar possesses an —OH group (hydroxyl) on the 2′ carbon. The deoxyribose sugar is missing the —OH group on the 2′ carbon. The *deoxy* part of the name deoxyribose indicates a "loss of oxygen" at position 2.

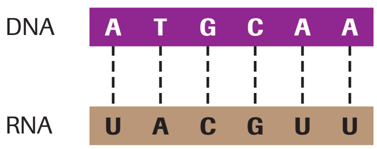


Figure 2
Base pairing of RNA with DNA during transcription. Notice that thymine does not exist in RNA but is substituted with uracil.

The Central Dogma

There are two main stages of gene expression, transcription and translation. In **transcription**, the genetic information is converted from a DNA sequence into **messenger RNA** (**mRNA**). In all cells, the mRNA carries the genetic information from the chromosome to the site of protein synthesis. In eukaryotic cells, which contain a nucleus, the mRNA carries the genetic information from the nucleus to the cytoplasm as it passes through the pores in the nuclear envelope.

transcription the process of converting DNA into messenger RNA

messenger RNA (mRNA) the product of transcription of a gene; mRNA is translated by ribosomes into protein

The second stage of gene expression is **translation**. During translation, the genetic information carried by the mRNA is used to synthesize a polypeptide chain.

translation the process of synthesizing a specific polypeptide as coded for by messenger RNA

The two-step process of transferring genetic information from DNA to RNA and then from RNA to protein is known as the central dogma of molecular genetics (**Figure 3**, next page). We will explore transcription and translation in more detail in this section. You

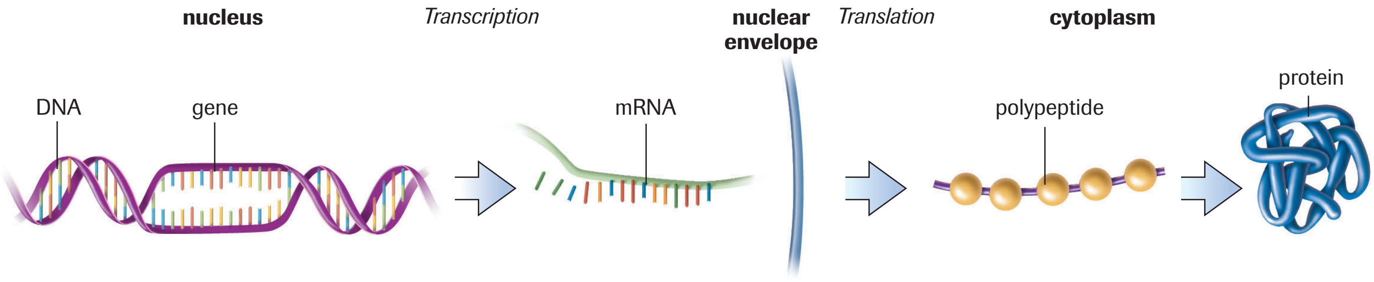


Figure 3
The central dogma of molecular genetics

will see that the sequence of nucleotides in a gene determines the sequence of amino acids in a polypeptide.

Transcription

During transcription, the DNA sequence of a gene is copied (transcribed) into the sequence of a single-stranded mRNA molecule.

Transcription is divided into three processes: initiation, elongation, and termination. During initiation, an enzyme called **RNA polymerase** binds to the DNA at a specific site near the beginning of the gene. During elongation, RNA polymerase uses the DNA as a template to build the mRNA molecule. During termination, the RNA polymerase passes the end of the gene and comes to a stop. The mRNA is then released from the template strand of DNA.

RNA polymerase enzyme that transcribes DNA

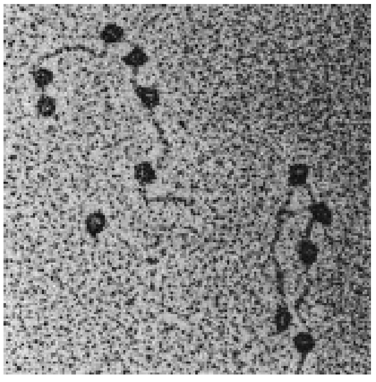


Figure 4
The RNA polymerase (dark circles) binds to the DNA strand and initiates transcription. Transcription occurs simultaneously at numerous locations along the DNA.

Initiation

Transcription starts when the RNA polymerase enzyme binds to the segment of DNA to be transcribed and opens the double helix. **Figure 4** shows an electron micrograph of this process. The RNA polymerase binds to the DNA molecule in front of the gene to be transcribed in a region called the **promoter**. In most genes, the promoter sequence contains a string of adenine and thymine bases that serves as the recognition site for RNA polymerase. The promoter indicates which DNA strand should be transcribed and where the RNA polymerase should start transcribing the DNA. Since the binding site of RNA polymerase only recognizes the promoter region, it can only bind in front of a gene.

promoter sequence of DNA that binds RNA polymerase in front of a gene

Elongation

Once the RNA polymerase binds to the promoter and opens the double helix, it starts building the single-stranded mRNA in the 5′ to 3′ direction. The promoter is not transcribed. The process of elongation of the mRNA molecule is similar to DNA replication. However, RNA polymerase does not require a primer and it copies only one of the DNA strands. The transcribed DNA strand is called the **template strand**. The mRNA sequence is complementary to the DNA template strand except that it contains the base uracil in place of thymine.

template strand the strand of DNA that the RNA polymerase uses as a guide to build complementary mRNA

Termination

Synthesis of the mRNA continues until RNA polymerase reaches the end of the gene. RNA polymerase recognizes the end of the gene when it comes to a stop signal called a **termination sequence**. Transcription stops and the newly synthesized mRNA disconnects from the DNA template strand. RNA polymerase is then free to bind to another promoter region and transcribe another gene. **Figure 5**, on the next page, summarizes the steps in transcription.

termination sequence sequence of bases at the end of a gene that signals the RNA polymerase to stop transcribing

(a) Initiation
RNA polymerase binds to DNA at a promoter.

(b) DNA double helix is unwound, exposing the template strand.

(c) Elongation
mRNA is synthesized using one strand of DNA as a template. mRNA is synthesized in the 5′ to 3′ direction.

(d) As elongation proceeds, RNA polymerase moves along DNA, synthesizing mRNA. DNA that has already been transcribed rewinds into a double helix.

(e) RNA polymerase reaches the termination sequence at end of gene.

(f) Termination
Transcription stops. mRNA and RNA polymerase are released.

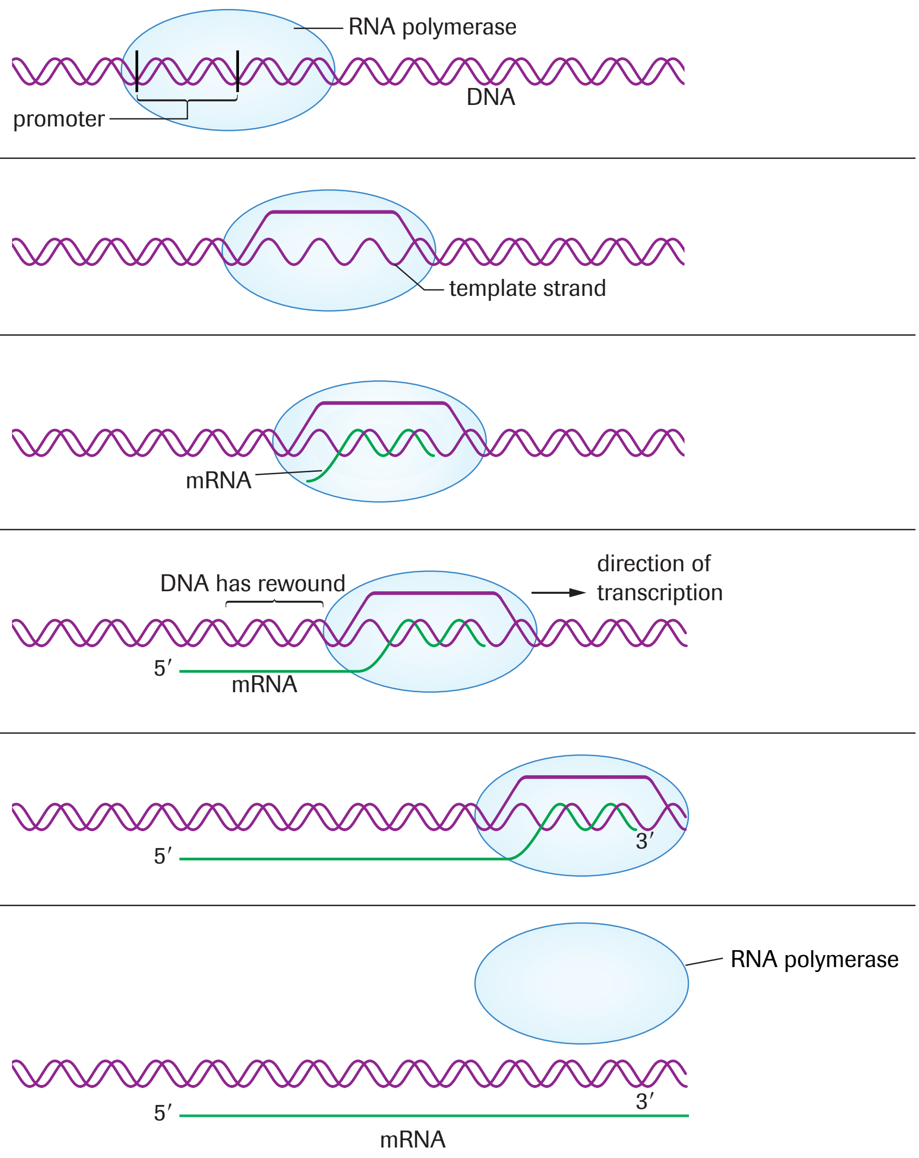


Figure 5
A summary of the process of transcription

Practice

1. A short fragment of a particular gene includes the following sequence of nucleotides:

   TACTACGGT

   Write out the corresponding mRNA transcript.

2. A short fragment of another gene includes the following sequence of nucleotides:

   ACCATAATATTACCGACCT TCG

   (a) Explain the purpose of the promoter region in transcription.
   (b) Copy the sequence into your notebook and circle the promoter region. Explain the rationale for your selection.

EXTENSION

Regulation of Transcription
This Audio Clip discusses the regulatory factors that control when and how much mRNA is transcribed from a given gene.

www.science.nelson.com GO

Translation

The second part of the central dogma of molecular biology (**Figure 3**, page 668) is the translation of the genetic information carried by mRNA into a chain of amino acids to form a polypeptide. Therefore, the process of translation involves protein synthesis, and it depends on the remarkable nature of the genetic code.

Only 20 amino acids are found in proteins. The DNA in a gene codes for these 20 amino acids by combinations of the four nitrogenous bases. During translation, the DNA code is read in groups of three nucleotides, called a **codon**. Each codon calls for a specific amino acid to be placed in the growing polypeptide chain. Codons can consist of any combination of the four nitrogenous bases, so there are 64 ($4^3 = 64$) possible different codons for the 20 different amino acids. The groups of three bases in both DNA and mRNA are both called codons, so it is important to clarify which code is being presented when writing out a genetic sequence. The remainder of this description will use mRNA codons. **Table 1** shows the mRNA codons. One of these codons (AUG) is the **start codon**, where translation begins. It also codes for the insertion of the amino acid methionine, so all polypeptide chains initially start with the methionine, but it may later be edited out. Three other codons (UAA, UAG, and UGA) do not code for amino acids and are called the **stop codons** because they cause protein synthesis to stop. The other 60 codons code for one of the 20 amino acids. Some amino acids have more than one codon; for example, both serine and leucine each have 6 different codons. **Table 2**, on the next page, lists the abbreviations for the amino acids to help you look them up in **Table 1**.

Like transcription, translation can be divided into the same three stages: initiation, elongation, and termination.

codon sequence of three bases in DNA or complementary mRNA that serves as a code for a particular amino acid

start codon specific codon (AUG) that signals the start of translation

stop codon specific codon that signals the end of translation

Table 1 Codons and Their Amino Acids

1st Base	2nd (middle) Base of a Codon				3rd Base
	U	C	A	G	
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA STOP UAG STOP	UGU Cys UGC Cys UGA STOP UGG Trp	U C A S
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A S
A	AUU Ile AUC Ile AUA Ile **AUG Met**	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A S
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A S

EXTENSION

Why Three Nucleotides per Codon?

Why are there always three nucleotides in a codon? Why not two or four? Listen to this Audio Clip to find out the reason behind the triplet code found in DNA and mRNA sequences.

www.science.nelson.com GO

Initiation

Initiation of translation occurs when a **ribosome** recognizes a specific sequence on the mRNA and binds to that site. In eukaryotes, the ribosome consists of two subunits, a large subunit and a small subunit (**Figure 6**, next page). The two subunits bind to the mRNA, clamping it between them. The ribosome then moves along the mRNA in the 5′ to 3′ direction, adding a new amino acid to the growing polypeptide chain each time it

ribosome an organelle composed of RNA and protein and located in the cytoplasm that carries out protein synthesis

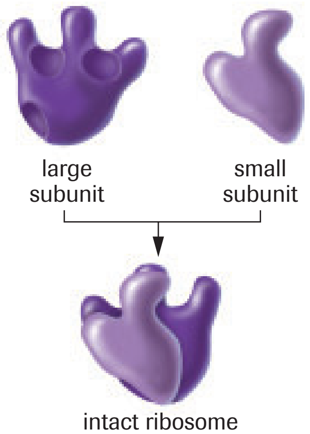


Figure 6
Ribosomes consist of a large subunit and a small subunit.

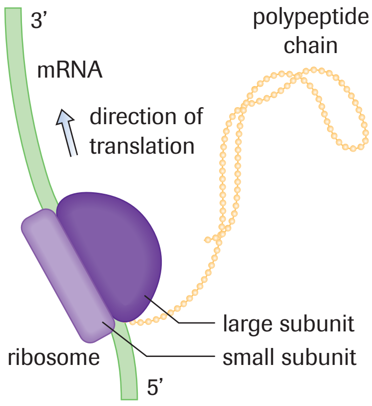


Figure 7
The large and small subunit of a ribosome work together to translate a strand of mRNA into a polypeptide. The polypeptide grows as the ribosome moves farther along the mRNA strand.

Table 2 Amino Acids and Their Abbreviations

Amino acid	Three-letter abbreviation
alanine	Ala
arginine	Arg
asparagine	Asn
aspartic acid	Asp
cysteine	Cys
glutamic acid	Glu
glutamine	Gln
glycine	Gly
histidine	His
isoleucine	Ile
leucine	Leu
lysine	Lys
methionine	Met
phenylalanine	Phe
proline	Pro
serine	Ser
threonine	Thr
tryptophan	Trp
tyrosine	Tyr
valine	Val

reads a codon (**Figure 7**). Ribosomes synthesize different proteins by associating with different mRNAs and reading their coding sequences.

A ribosome must begin reading the coding sequence at the correct place in the mRNA, or it will misread all the codons. The first codon that it recognizes is the start codon AUG. Binding to the start codon ensures that the ribosome translates the genetic code using the reading frame of the mRNA molecule. It is critical that the mRNA be positioned in the ribosome in its reading frame so that the genetic code is translated into the correct sequence of amino acids.

Once the ribosome has bound the mRNA, how does it get the amino acids that correspond to the codon? This job falls to a second type of RNA molecule known as **transfer RNA** (**tRNA**). At one end of the tRNA there is a sequence of three bases, the **anticodon**, that is complementary to the codon of the mRNA. The opposite end carries the corresponding amino acid (**Figure 8**, next page). For example, if the mRNA has the codon UAU, the complementary base sequence of the anticodon is AUA, and the tRNA would carry the amino acid tyrosine. Check **Table 1** to find the mRNA codon and prove to yourself that it calls for tyrosine. Every tRNA carries only one specific amino acid, which means that at least 20 different tRNAs are required. Recall that there are 64 possible codons. In reality, anywhere from 20 to 64 types of tRNA molecules are available, depending on the organism.

transfer RNA (**tRNA**) the form of RNA that delivers amino acids to a ribosome during translation

anticodon group of three complementary bases on tRNA that recognizes and pairs with a codon on the mRNA

Practice

3. Transcribe the following sequence of DNA into mRNA.
   TACGGATTTCTCCGCAAATTAGGG
4. Translate the following mRNA sequence into an amino acid sequence.
   5′-AUGCCCUCUAUUCCGGGAAGAUAG-3′
5. How many nucleotides are necessary in the DNA to code for the following sequence of amino acids?
   Leu-Tyr-Arg-Trp-Ser

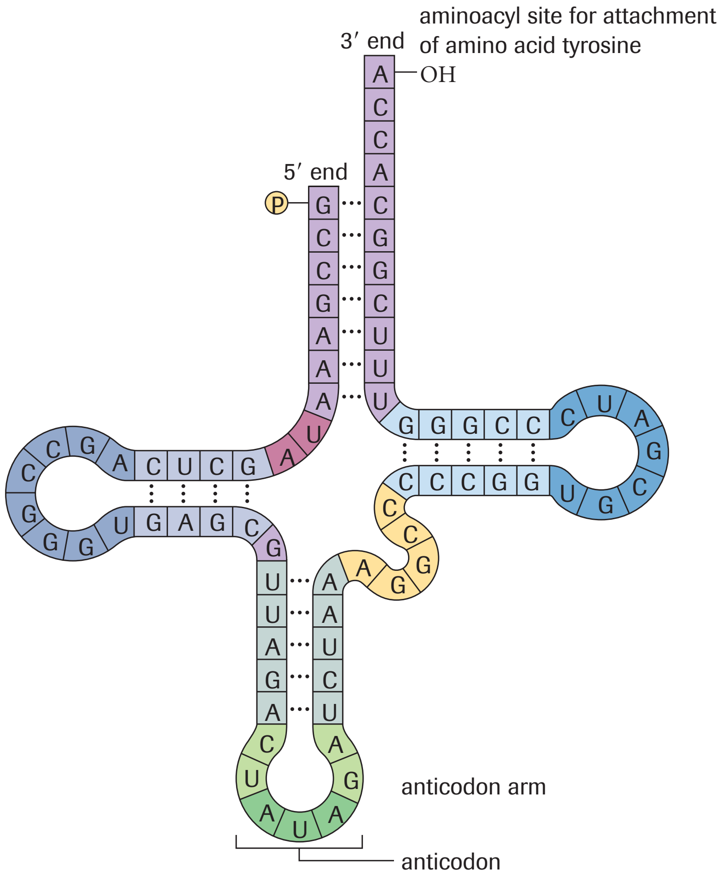


Figure 8
The tRNA molecule has a cloverleaf structure. The molecule folds to form this structure because of hydrogen bonding. The anticodon is located on the anticodon arm and the amino acid is covalently bound to the adenine nucleotide at the 3′ end (aminoacyl). In this case, the amino acid that would be added is tyrosine because the anticodon is AUA.

Elongation

The first codon that is recognized by the ribosome is the start codon AUG. The AUG codon also codes for methionine, so every protein initially starts with the amino acid methionine. The ribosome has two sites for tRNA to attach: the A (aminoacyl) site and the P (peptidyl) site. The tRNA with the anticodon complementary to the start codon enters the P site, as shown in **Figure 9 (a)**. The next tRNA carrying the required amino acid enters the A site, as shown in **Figure 9 (b)**. In **Figure 9 (c)**, a peptide bond has formed between the methionine and the second amino acid, alanine. The ribosome has shifted over one codon so that the second tRNA is now in the P site. This action has released the methionine-carrying tRNA from the ribosome and allowed a third tRNA to enter the empty A site. The process is similar to a tickertape running through a ticker-tape machine, except that the ribosome "machine" moves along the mRNA "tickertape." The tRNAs that have been released are recycled in the cell cytoplasm by adding new amino acids to them. The process continues until the entire code of the mRNA has been translated and the ribosome reaches a stop codon, as shown in **Figures 9 (d)** and **(e)**.

DID YOU KNOW ?

From DNA to Protein

The discovery of the relationship between DNA, mRNA, ribosomes, tRNA, and protein was the result of numerous scientists working on separate pieces of the puzzle. Watch an online animation of their studies.

www.science.nelson.com GO

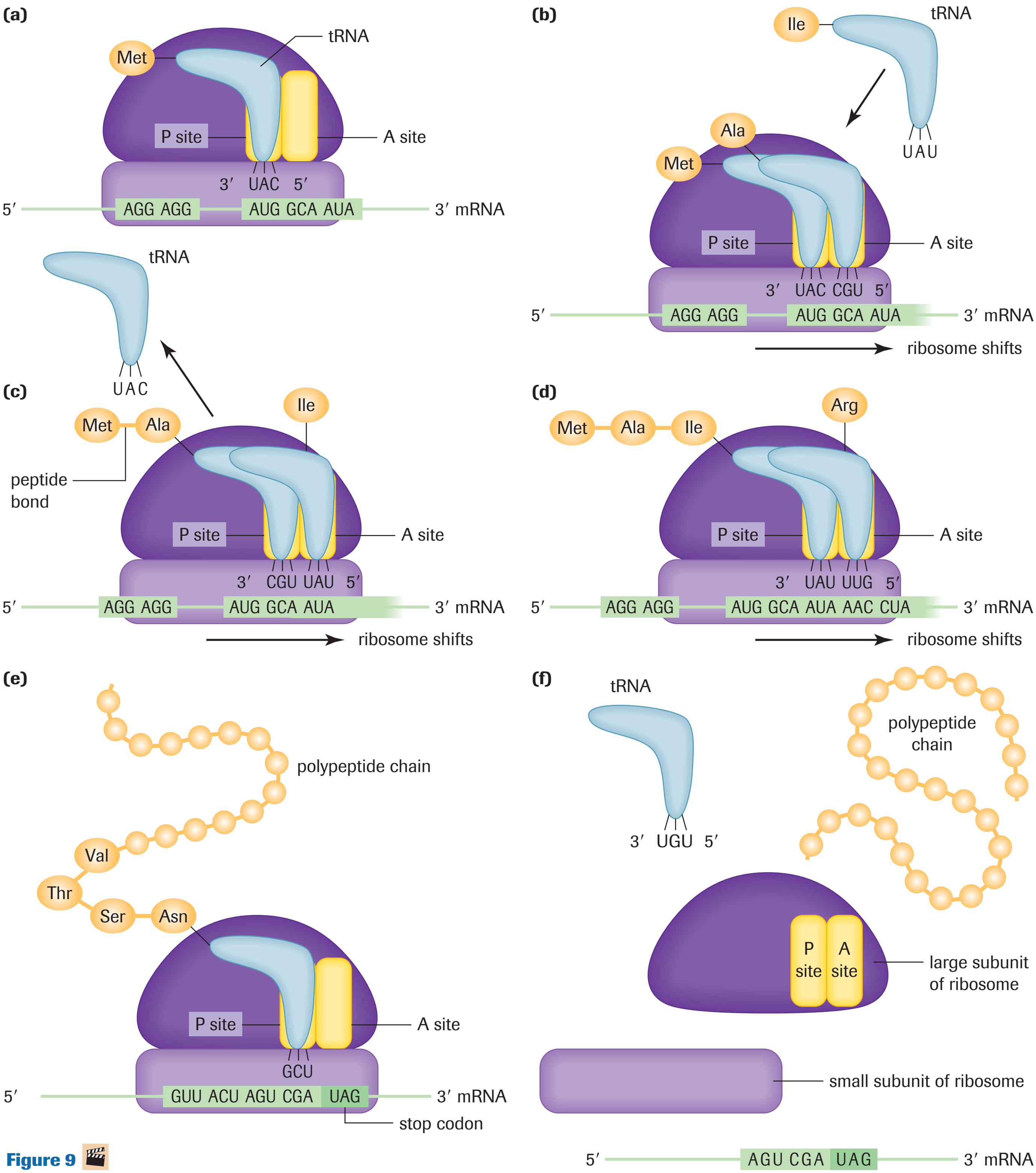


Figure 9
Protein synthesis
(a) The first tRNA that is brought into the P site carries methionine because the start codon is AUG.
(b) The second tRNA enters the A site.
(c) A peptide bond forms between methionine and alanine. The ribosome shifts one codon over and the next tRNA brings in the appropriate amino acid into the A site.
(d) The ribosome moves the mRNA and another amino acid is added to the chain.
(e) The process is repeated until the ribosome reaches a stop codon for which no tRNA exists.
(f) A release-factor protein helps break apart the ribosome–mRNA complex, releasing the polypeptide chain.

Termination

Eventually, the ribosome reaches one of the three stop codons: UGA, UAG, or UAA. Since these three codons do not code for an amino acid, there are no corresponding tRNAs. A protein known as a release factor recognizes that the ribosome has stalled and helps release the polypeptide chain from the ribosome. As shown in **Figure 9 (f)**, on the previous page, the two subunits of the ribosome now fall off the mRNA and translation stops.

LAB EXERCISE 20.A

Report Checklist

- ○ Purpose
- ○ Problem
- ○ Hypothesis
- ○ Prediction
- ○ Design
- ○ Materials
- ○ Procedure
- ○ Evidence
- ● Analysis
- ○ Evaluation
- ○ Synthesis

Synthesis of a Protein

In this activity, you are provided with a DNA nucleotide sequence that codes for a hypothetical protein. The code is given in three fragments. This DNA code is from a eukaryotic cell so in the mRNA transcript there are extra codons called introns. Eukaryotic cells cut these sequences out of the mRNA before it leaves the nucleus, so the codons are transcribed but are not translated.

In this exercise, you will transcribe the three pieces of DNA code into mRNA and identify the beginning fragment, the middle fragment, and the end fragment. In addition, you will remove the intron segment and translate the mRNA into the protein.

Procedure

1. Copy each of the following sequences onto a separate piece of paper. (*Hint:* Turn your paper so you can write the sequence out along the horizontal length of the paper. Leave room below each sequence to write your mRNA sequence directly below.)

   Sequence A
   CTCGCGCCGAAACTTCCCTCCTAAACGTTCAAC
   CGGTTCTTAATCCGCCGCCAGGGCCCC

   Sequence B
   CGTAACAACTTGTTACAACATGGTCATAAACGTCA
   GATGGTCAATCTCTTAATGACT

   Sequence C
   TACAAACATGTAAACACACCCTCAGTGGACCAA
   CTCCGCAACATAAACCAAACACCG

2. Divide the sequences into triplets (codons) by putting a slash between each group of three bases.
3. Transcribe the DNA into mRNA.
4. Identify the middle, end, and beginning sequence. Use your knowledge of start and stop codons to help you figure it out. (*Hint:* You will need to examine the codons that start and end a fragment.)
5. Remove codons 24 to 51, including codon 51. These codons are the intron, or extra codons, found in this DNA segment.
6. Translate the mRNA into protein using the genetic code.

Analysis

(a) Which fragment was the beginning fragment? How do you know?

(b) Which fragment was the end fragment? How do you know?

(c) Codons 24 to 51 represent an intron. If the introns were not cut out of the mRNA before it leaves the nucleus and attaches to a ribosome, what would happen to the protein structure? Is it likely that this protein would still perform the same function? Explain your answer.

(d) How many amino acids does this protein contain?

(e) Is this genetic sequence eukaryotic or prokaryotic? How do you know?

(f) If you worked backward, starting with the amino acid sequence of the protein, would you obtain the same DNA nucleotide sequence? Why or why not?

(g) Provide the anticodon sequence that would build this protein.

INVESTIGATION 20.1 *Introduction*

Protein Synthesis and Inactivation of Antibiotics

Each protein has a specific function. Its presence or absence in a cell may make the difference between life and death. Bacteria that carry an ampicillin-resistance gene produce a protein that inactivates the antibiotic ampicillin. What happens when they are grown on ampicillin-rich media? This investigation allows you to observe the effects of the presence and function of a specific gene.

Report Checklist

- ● Purpose
- ○ Problem
- ● Hypothesis
- ● Prediction
- ● Design
- ○ Materials
- ○ Procedure
- ● Evidence
- ● Analysis
- ● Evaluation
- ● Synthesis

To perform this investigation, turn to page 695.

SUMMARY *Gene Expression*

Table 3 Summary of Transcription

Initiation
• Initiation of transcription starts when the RNA polymerase binds to the promoter region of the gene to be transcribed. • The DNA is unwound and the double helix is disrupted.
Elongation
• A complementary messenger RNA (mRNA) molecule is synthesized in the 5′ to 3′ direction, using one strand of DNA as a template. • Adenine (A) bases in the DNA are paired with uracil (U) in the mRNA. • Transcription continues until the RNA polymerase reaches a termination sequence.
Termination
• When the RNA polymerase comes to a termination sequence, it falls off the DNA molecule. • The mRNA separates from the DNA.

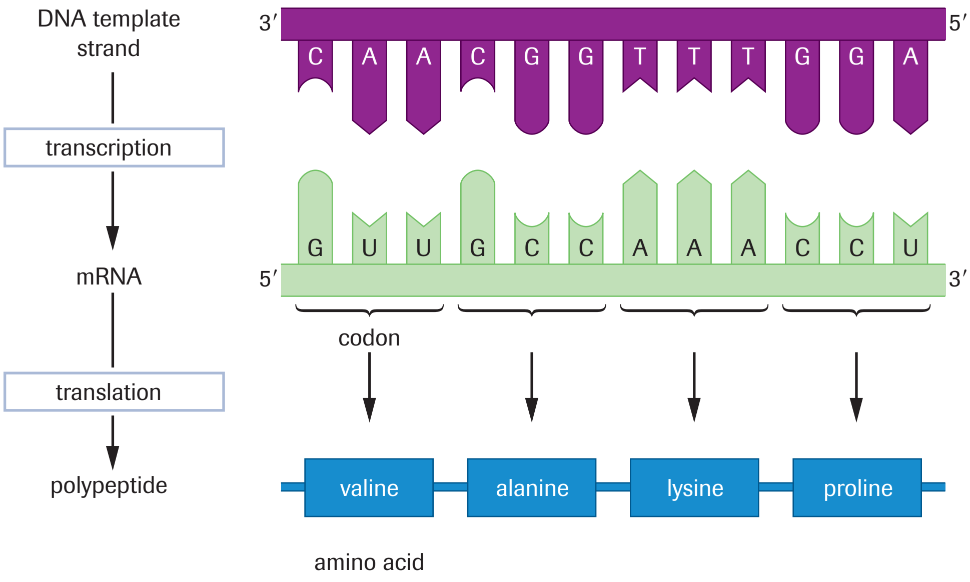


Figure 10
An overview of gene expression

Table 4 Summary of Translation

Initiation
• Ribosome subunits (large and small) bind to the mRNA transcript, sandwiching the mRNA between them.
• The ribosome moves along the mRNA, reading the codons.
• Translation begins when the ribosome reaches the start codon, AUG.
Elongation
• Through the genetic code, each codon specifies a particular one of the 20 amino acids that make up polypeptides.
• Transfer RNA (tRNA) molecules have an anticodon that is complementary to the codon in the mRNA. The tRNA carries the amino acid specified by the codon.
• The ribosome contains two sites, the A (aminoacyl) site and the P (peptidyl) site.
• When the start codon is in the P site, the first tRNA delivers methionine. Since the start codon codes for methionine, all polypeptides initially start with this amino acid.
• The second codon of the mRNA is exposed at the same time in the A site. When the tRNA delivers the second amino acid, a peptide bond is formed between the two amino acids.
• The ribosome shifts over one codon. The tRNA that delivered the methionine is released from the P site.
• When the ribosome shifts, the tRNA containing the growing polypeptide moves to the P site. A third amino acid, specified by the third codon, is brought in to the A site by the next tRNA. A peptide bond is formed between the second and third amino acid.
• Amino acids continue to be added to the polypeptide until a stop codon is read in the A site.
Termination
• The stop codons are UAG, UGA, and UAA. At this point the ribosome stalls.
• A protein known as the release factor recognizes that the ribosome has stalled and causes the ribosome subunits to disassemble, releasing the mRNA and newly formed polypeptide.

Section 20.2 Questions

1. State the central dogma of molecular genetics.
2. Describe the role of the following molecules in gene expression: ribosomes, mRNA, tRNA.
3. The genetic code is read in groups of three nucleotides called codons. Explain why reading the code in pairs of nucleotides is not sufficient.
4. The following is the sequence of a fragment of DNA:

   GGATCAGGTCCATAC

   Transcribe this sequence into mRNA.
5. Using the genetic code, decipher the following mRNA sequence:

   5′ - AUGGGACAUUAUUUUGCCCGUUGUGGU - 3′
6. The amino acid sequence for a certain peptide is Leu–Tyr–Arg–Trp–Ser. How many nucleotides are necessary in the DNA to code for this peptide?
7. Identify which step in transcription would be affected and predict what would happen in each situation:
   (a) The termination sequence of a gene is removed.
   (b) RNA polymerase fails to recognize the promoter.
8. Construct a table to compare the processes of replication and transcription. Remember to consider both similarities and differences.
9. Distinguish between the following terms:
   (a) P site and A site
   (b) codon and anticodon
   (c) start and stop codon
   (d) DNA and RNA
10. Identify which of the following selections correctly lists the anticodons for the amino acids threonine, alanine, and proline:

A.	ACU	GCU	CCA
B.	ACT	GCT	CCA
C.	TGA	CGA	GGT
D.	UGA	CGA	GGU

11. Errors are occasionally made during the process of transcription. Explain why these errors do not always result in an incorrect sequence of amino acids. Describe at least two examples to illustrate your answer.

DNA and Biotechnology 20.3

Carpenters require tools such as hammers, screwdrivers, and saws, and surgeons require scalpels, forceps, and stitching needles. The tools of the molecular biologist are living biological organisms or biological molecules. Using these tools, scientists can treat specific DNA sequences as modules and move them from one DNA molecule to another, forming **recombinant DNA**. Research in exploring and using this type of biotechnology has led to exciting new advances in biological, agricultural, and medical technology. Biotechnology research has also found ways to introduce specific DNA sequences into a living cell. For example, the gene that encodes insulin has been introduced into bacterial cells so that they become living factories producing this vital hormone. The introduction and expression of foreign DNA in an organism is called **genetic transformation**. In this section, you will explore some of the key tools used by molecular geneticists in producing recombinant DNA and genetically transformed organisms.

recombinant DNA fragment of DNA composed of sequences originating from at least two different sources

genetic transformation introduction and expression of foreign DNA in a living organism

DNA Sequencing

Before a DNA sequence can be used to make recombinant DNA or to transform an organism, the scientist or technician must first identify and isolate a piece of DNA containing that sequence. One of the tools used to do this is DNA sequencing. DNA sequencing determines the exact sequence of base pairs for a particular DNA fragment or molecule. In 1975, the first DNA sequencing techniques were simultaneously developed by Frederick Sanger and his colleagues and by Alan Maxim and Walter Gilbert. Sanger's technique relied on first replicating short segments of DNA that terminate due to a chain-terminating nucleotide. Four separate reaction tubes are run, each with a chain-terminating nucleotide incorporating a different base (i.e., A, T, G, and C). The various lengths of DNA segments are then separated by loading and running the contents of the tubes on a sequencing gel (**Figure 1**). Because the end nucleotide of each segment is chain-terminating, its base is already known. Consequently, the sequence can be read directly from the gel in ascending order (shortest to longest segments). The sequence of the strand is written along the edge of the gel diagram, starting from the bottom where the shortest strands have travelled. This method is comparatively slow and can only sequence short fragments of DNA.

DNA can also be sequenced in a test tube using isolated segments of DNA. This technique depends on a primer, DNA polymerase, and the four DNA nucleotides, each of which is labelled with a specific dye. The complementary strand is built from these dye-labelled nucleotides. The nucleotides in the synthesized strand can then be identified by their colours, allowing the original strand sequence to be deduced according to the rules of complementary base pairing.

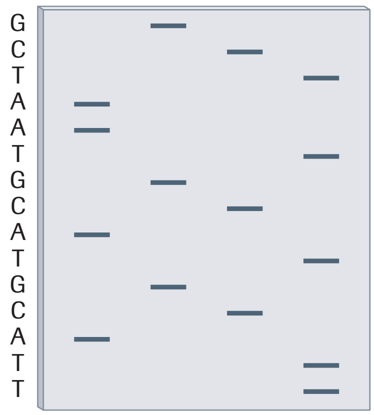


Figure 1
A sequencing gel is a matrix containing many small spaces. The DNA fragments are charged and will move towards one pole of an electric field. Smaller DNA fragments move through the spaces more quickly than larger fragments and are found at the bottom of the gel. The larger fragments will remain towards the top of the gel. The resulting ladder of fragments can be read, giving the sequence of the initial DNA fragment.

WWW ***WEB Activity***

Simulation—Electrophoresis

Electrophoresis is an important tool in molecular biology. In addition to nucleic acids, it is also used to separate proteins from a mixture. Electrophoresis of nucleic acids and proteins depend on the similar factors. In this Virtual Biology Lab, you will perform polyacrylamide gel electrophoresis (PAGE) to identify proteins involved in the biochemistry of shell colour in an extinct organism.

www.science.nelson.com GO

Figure 2
Dr. Judith Hall

WEB Activity

Canadian Achievers—Researchers in Human Genetic Disorders

Advances in biotechnology have led a greater understanding of many human genetic disorders. These advances have involved many research teams working together, either directly or by publishing their work in peer-reviewed articles. The following list shows some Canadians who are among the researchers making important contributions:

- Dr. Michael Hayden, University of British Columbia: Huntington disease
- Dr. Lap-Chee Tsui, Hospital for Sick Children, Toronto: cystic fibrosis
- Dr. Judith Hall, University of British Columbia: cystic fibrosis
- Dr. Christine Bear, University of Toronto: cystic fibrosis
- Dr. Ron Warton, Hospital for Sick Children, Toronto: Duchenne muscular dystrophy

Go to the Nelson Web site to find more information on the work of these people. After you have completed reading this material, write a short paragraph that describes your view on the importance of genetic research. Defend your position.

www.science.nelson.com GO

DID YOU KNOW ?

Genome Facts

On February 15, 2001, scientists from the Human Genome Project and Celera Genomics confirmed that there were approximately 30 000 genes in the human genome—a number far less than the original estimate of 120 000. This was determined using two different DNA sequencing techniques.

Other Facts

- 99.9 % of the nitrogenous base sequences is the same in all humans.
- Only 5 % of the genes contains the instructions for producing functional proteins; the remaining 95 % does not have any known function.
- A worm has approximately 18 000 genes; a yeast cell has about 6000.

The Human Genome Project

In a series of meetings held in the mid-1980s, plans were developed to begin the process of producing maps of the entire genetic makeup of a human being. The international project began in the United States in October 1990 with James Watson, of Nobel Prize fame, as one of the first directors. The human genome consists of approximately 30 000 genes, with the 23 pairs of chromosomes containing an estimated 3 billion pairs of nucleotides. Constructing the genome map involved using mapping techniques (similar to those you read about in Chapter 19) and DNA sequencing technology. When the project began, only about 4500 genes had been identified and sequenced. The collaborative efforts of many scientists from numerous countries and rapid improvements in sequencing techniques helped complete the gene map by June 2000 (**Table 1**).

Table 1 Milestones in Genome Mapping

Milestone	Date
human chromosome 22 completed (the first chromosome to be mapped)	December 1999
Drosophila genome completed	March 2000
human chromosomes 5, 16, 19 mapped	April 2000
human chromosome 21 completely mapped	May 2000
human genome completely mapped	June 2000

A DNA sequencing technique based on the one developed by Sanger was the most common method used in the project. In this technique, pieces of DNA are replicated and changed so that the fragments, each ending with one of the four nucleotides, can be detected by a laser. Automated equipment can then determine the exact number of nucleotides in the chain. A computer is used to combine the huge amount of data and reconstruct the original DNA sequence.

Prior to the Human Genome Project, the genes for hereditary disorders such as cystic fibrosis, muscular dystrophy, and Huntington disease had been identified. The aim of the project is to add to this list so that new drugs and genetic therapies can be developed to

mini *Investigation* — *Examining the Human Genome*

In this activity, you will go to an online map of the human genome. On the map, you will find diagrams containing information about every chromosome in the genome. The magenta and green regions on the diagrams reflect the unique patterns of light and dark bands seen on human chromosomes that have been stained for viewing through a light microscope. The red region represents the centromere or constricted portion of the chromosome. On other chromosome diagrams, you will see yellow regions that mark chromosomal areas that vary in staining intensity. The chromatin in these areas is condensed and sometimes known as heterochromatin, meaning “different colour.” Some diagrams have yellow regions overlaid by thin horizontal magenta lines. This colour pattern indicates variable regions called stalks that connect very small chromosome arms (satellites) to the chromosome.

www.science.nelson.com GO

Go to the Nelson Web site, and follow the link for Mini Investigation: Examining the Human Genome. On the genome map, click on each chromosome diagram to discover the traits and disorders located on that chromosome. For example, **Figure 3** shows traits and disorders that are found on chromosome 20.

Touch each chromosome pair to find the number of genes mapped on that chromosome.

Use the information you find to answer the questions below.

(a) Which chromosome pair contains the greatest number of genes?

(b) Which chromosome contains the fewest genes?

(c) Estimate the size of the human genome. Show how you calculated your estimate.

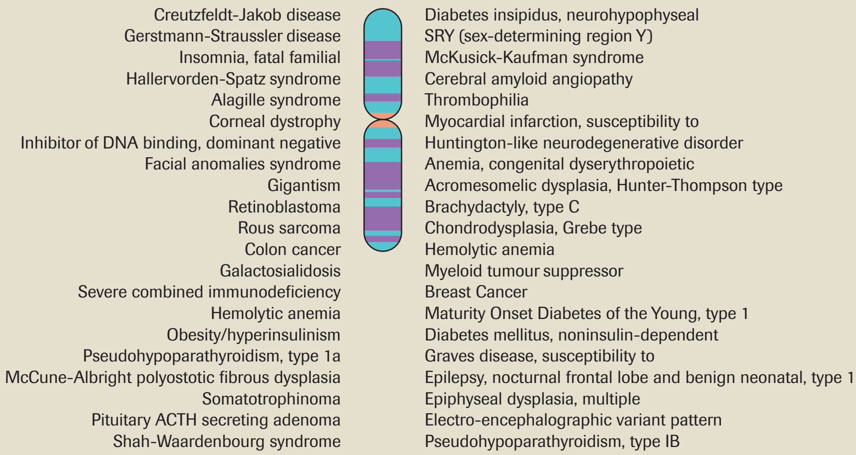


Figure 3
Although chromosome 20 is one of the smallest chromosomes, it has a great number of genes.

combat genetic disorders. The project also may open a Pandora’s box of ethical questions, legal dilemmas, and societal problems. Who will own or control the information obtained and how will we prevent potential misuse of the data?

Enzymes and Recombinant DNA

As you have seen in this section, DNA sequencing is one way to identify specific segments of DNA. Another way is by creating genetic linkage maps, as you saw in Chapter 18. Once a particular segment of DNA has been identified, molecular biologists may use enzymes to isolate that segment or modify it. The DNA fragment may then be used to create recombinant DNA or be transferred to another organism. We will review some of the most commonly used enzymes.

CAREER CONNECTION

Biotechnologist
Biotechnologists are involved in improving and developing processes and products used in agriculture, health care, and the chemical industry. A biotechnologist needs specialized knowledge of biochemistry, microbiology, and molecular genetics. Find out more about opportunities in this field.

www.science.nelson.com GO

restriction endonuclease an enzyme that cuts double-stranded DNA into fragments at a specific sequence; also known as a restriction enzyme

recognition site a specific sequence within double-stranded DNA that a restriction endonuclease recognizes and cuts

palindromic reading the same backwards and forwards

Restriction Endonucleases

Restriction endonucleases, otherwise known as restriction enzymes, are like molecular scissors that can cut double-stranded DNA at a specific base-pair sequence. Each type of restriction enzyme recognizes a particular sequence of nucleotides that is known as its **recognition site**. Molecular biologists use these enzymes to cut DNA in a predictable and precise way. Most recognition sites are four to eight base pairs long and are usually characterized by a complementary **palindromic** sequence (**Table 2**). For example, look at the restriction enzyme *Eco*RI. This sequence is palindromic because both strands have the same base sequence when read in the 5′ to 3′ direction.

Table 2 Restriction Enzymes and Their Recognition Sites

Microorganism of origin	Enzyme	Recognition site	After restriction enzyme digestion
Escherichia coli	*Eco*RI	5′-GAATTC-3′ 3′-CTTAAG-5′	5′-G AATTC-3′ 3′-CTTAA G-5′
Serratia marcescens	*Sma*I	5′-CCCGGG-3′ 3′-GGGCCC-5′	5′-GGG CCC-3′ 3′-CCC GGG-5′
Arthrobacter luteus	*Alu*I	5′-AGCT-3′ 3′-TCGA-5′	5′-AG CT-3′ 3′-TC GA-5′
Streptomyces albus	*Sal*I	5′-GTCGAC-3′ 3′-CAGCTG-5′	5′-G TCGAC-3′ 3′-CAGCT G-5′
Haemophilus parainfluenzae	*Hind*III	5′-AAGCTT-3′ 3′-TTCGAA-5′	5′-A AGCTT-3′ 3′-TTCGA A-5′

DID YOU KNOW ?

Maps and Libraries

Restriction endonucleases are also used to create genetic maps and libraries. Go to the Nelson Web site for information on these applications.

www.science.nelson.com GO

Figure 4 shows the action of the restriction enzyme *Eco*RI. *Eco*RI scans a DNA molecule and stops when it is able to bind to its recognition site. Once bound to the site, it cuts the bond between the guanine and adenine nucleotides on each strand. At the end of each cleavage site, one strand is longer than the other and has exposed nucleotides that lack complementary bases. The overhangs produced by the exposed DNA nucleotides are called **sticky ends**. *Eco*RI always cuts between the guanine and the adenine nucleotide on each strand. Since A and G are at opposite ends of the recognition site on each of the complementary strands, the result is the overhang, or sticky end, at each cleavage site.

sticky ends fragment ends of a DNA molecule with short single-stranded overhangs, resulting from cleavage by a restriction enzyme

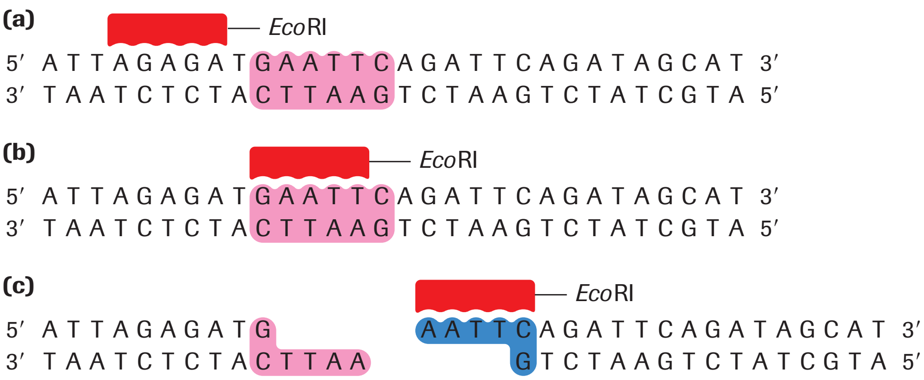


Figure 4
Cleavage of DNA sequence using restriction enzyme *Eco*RI.
(a) *Eco*RI scans the DNA molecule.
(b) *Eco*RI binds to the recognition site.
(c) *Eco*RI cuts between the guanine and adenine nucleotides, producing two fragments with complementary ends.

Not all restriction endonucleases produce sticky ends. For example, the restriction endonuclease *Sma*I produces **blunt ends**, which means that the ends of the DNA fragments are fully base paired (**Table 2**). Since *Sma*I cuts between the cytosine and guanine nucleotides and since these nucleotides are directly opposite each other in their complementary strands, the result is a blunt cut without sticky ends.

blunt ends fragment ends of a DNA molecule that are fully base paired, resulting from cleavage by a restriction enzyme

Restriction endonucleases that produce sticky ends are a generally more useful tool to molecular biologists than those that produce blunt ends. Sticky-end fragments can be joined more easily through complementary base pairing to other sticky-end fragments that were produced by the same restriction endonuclease. However, this is not always possible. To create recombinant DNA, molecular biologists choose restriction enzymes that will not cut in the middle of the DNA sequence of interest. For example, if the goal is to create recombinant DNA containing a particular gene, you would avoid using a restriction enzyme that cuts within the sequence of that gene.

Restriction enzymes are named according to the bacteria they come from. Generally speaking, the first letter is the initial of the genus name of the organism. The second and third letters are usually the initial letters of the species name. The fourth letter indicates the strain, while the numerals indicate the order of discovery of that particular enzyme from that strain of bacteria.

Learning Tip

Restriction enzymes are named according to specific rules. For example, the restriction enzyme *Bam*HI is named as follows:

- *B* represents the genus *Bacillus.*
- *am* represents the species *amyloliquefaciens.*
- *H* represents the strain.
- *I* means that it was the first endonuclease isolated from this strain.

Practice

1. The following sequence of DNA was digested with the restriction endonuclease *Sma*I:

   5′-AATTCGCCCGGGATATTACGGATTATGCATTATCCGCCCGGGATATTTTAGCA-3′

   3′-TTAAGCGGGCCCTATAATGCCTAATACGTAATAGGCGGGCCCTATAAAATCGT-5′

   *Sma*I recognizes the sequence CCCGGG and cuts between the C and the G.

   (a) Copy this sequence into your notebook and clearly identify the location of the cuts on it.

   (b) How many fragments will be produced if *Sma*I digests this sequence?

   (c) What type of ends does *Sma*I produce?
2. *Hin*dIII recognizes the sequence AAGCTT and cleaves between the two A's. What type of end is produced by cleavage with *Hin*dIII?
3. Explain why restriction endonucleases are considered to be molecular tools.
4. Copy the following sequence of DNA into your notebook. Write out the complementary strand. Clearly identify the palindromic sequences by circling them.

   GCGCTAAGGATAGCATTCGAATTCCCAATTAGGATCCTTTAAAGCTTATCC

DID YOU KNOW ?

The First Restriction Enzyme

The first restriction endonuclease, *Hin*dIII, was identified in 1970 by Hamilton Smith at John Hopkins University. Smith received the Nobel Prize in 1978 for his discovery. Since then, more than 2500 restriction endonucleases have been identified.

Methylases

Methylases are enzymes that can modify a restriction enzyme recognition site by adding a methyl ($–CH_3$) group to one of the bases in the site (**Figure 5**). Methylases are important tools in recombinant DNA technology. They protect a gene fragment from being cut in an undesired location.

methylase an enzyme that adds a methyl group to one of the nucleotides found in a restriction endonuclease recognition site

Like restriction enzymes, methylases were first identified in bacterial cells. Methylases are used by a bacterium to protect its DNA from digestion by its own restriction enzymes. In bacteria, restriction enzymes provide a crude type of immune system. In fact, the term *restriction* comes from early observations that these enzymes appeared to restrict the infection of *E. coli* cells by viruses known as bacteriophages. The restriction enzymes bind to recognition sites in the viral DNA and cut it, making it useless. Eukaryotic cells also contain methylases. However, in eukaryotes methylation usually occurs in order to inactivate specific genes.

CH_3
G A A T T C
C T T A A G
CH_3

Figure 5
At a methylated *Eco*RI site, *Eco*RI restriction enzyme is no longer able to cut.

Eukaryotic Methylation

Methylases in eukaryotes are connected with the control of transcription. In addition, approximately 2 % of mammalian ribosomal RNA is methylated after it is transcribed.

DNA Ligase

To create recombinant DNA, pieces of DNA from two sources must be joined together. Using restriction enzymes and methylases, molecular geneticists can engineer fragments of DNA that contain the specific nucleotide sequences they want. These segments of DNA are then joined together by DNA ligase. If two fragments have been generated using the same restriction enzyme, they will be attracted to each other at their complementary sticky ends. Hydrogen bonds will form between the complementary base pairs. DNA ligase then joins the strands of DNA together (**Figure 6**).

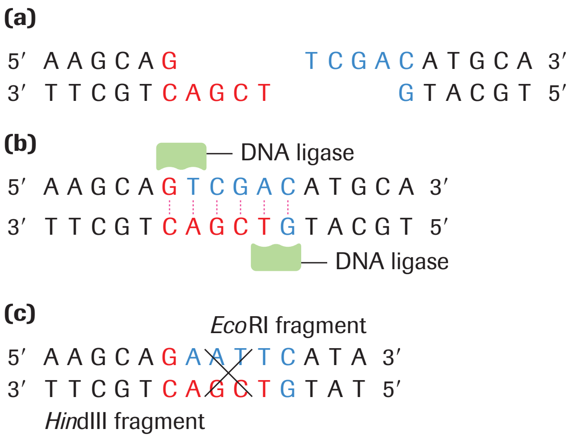


Figure 6
DNA ligase is able to join complementary sticky ends produced by the same restriction enzyme via a condensation reaction.
(a) Complementary sticky ends produced by *Hin*dIII
(b) Hydrogen bonds form between complementary bases. DNA ligase creates bonds between nucleotides in the DNA backbones.
(c) If fragments are not complementary, then hydrogen bonds will not form.

Taq DNA Polymerase and the Polymerase Chain Reaction

In 1985, American scientist Kary Mullis invented a biotechnology technique called the **polymerase chain reaction** (**PCR**). PCR allows scientists to make billions of copies of pieces of DNA from extremely small quantities of DNA. The reaction depends on the special property of *Taq* polymerase. In nature, *Taq* DNA polymerase is found in the bacterium *Thermos aquaticus*, which lives at extremely high temperatures. Like all the DNA polymerases, *Taq* DNA polymerase synthesizes DNA during replication. As you have learned previously, enzymes have an optimum temperature range in which they function. One adaptation that allows *Thermos aquaticus* to survive at high temperatures is that its DNA polymerase is stable at much higher temperatures than DNA polymerases from other organisms. Mullis used the heat-stable property of *Taq* polymerase in his PCR technique.

polymerase chain reaction (**PCR**) a technique for amplifying a DNA sequence by repeated cycles of strand separation and replication

To prepare for PCR, the following materials are placed together in a small tube: *Taq* polymerase, the DNA to be copied, a large quantity of the four deoxynucleotides (A, T, G, and C), and short primers. The tube is then inserted into a PCR machine. PCR involves four simple steps (**Figure 7**).

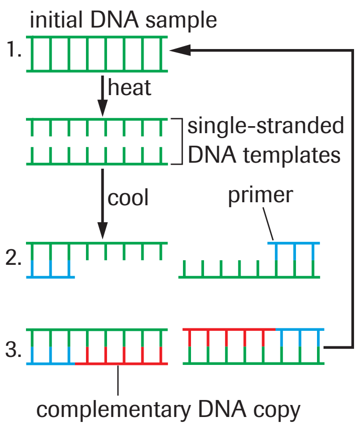


Figure 7
Steps in the PCR
1. The mixture is heated to a temperature high enough to break the hydrogen bonds in the double helix of the DNA and separate the strands. This forms single-stranded DNA templates.
2. The mixture is cooled, and the primers form hydrogen bonds with the DNA templates.
3. *Taq* polymerase synthesizes a new stand of DNA from the DNA template by complementary base pairing, starting at each primer.
4. The cycle of heating and cooling is repeated many times.

Each PCR cycle doubles the number of DNA molecules. After just 10 cycles there are 2^{10} (over two million) copies of the DNA template. Since scientists can use PCR to synthesize many identical copies from a very small sample of DNA, this technology has led to many advances in medicine, evolutionary biology, genetic engineering, and forensic science. Mullis was awarded the Nobel Prize in Chemistry in 1993 for his invention.

INVESTIGATION 20.2 *Introduction*

Restriction Enzyme Digestion of Bacteriophage DNA

Using restriction enzymes and electrophoresis, molecular biologists are able to excise and isolate target sequences from DNA. How would the banding patterns compare if the same fragment of DNA were digested with different restriction enzymes? In this investigation, you will conduct electrophoresis of bacteriophage DNA that has been digested with restriction enzymes.

Report Checklist

● Purpose	● Design	● Analysis
○ Problem	○ Materials	● Evaluation
● Hypothesis	○ Procedure	○ Synthesis
● Prediction	● Evidence	

To perform this investigation, turn to page 696.

Transformation

So far, you have seen that mapping and sequencing can be used to identify the relative position and nucleotide sequence of genes in a DNA molecule. Using various enzymes, scientists can isolate DNA fragments containing a gene or genes. Multiple copies of the fragment can be prepared using PCR. The DNA fragment may also be joined (annealed) to other DNA fragments.

In genetics, transformation is any process by which foreign DNA is incorporated into the genome of a cell. A **vector** is the delivery system used to move the foreign DNA into a cell. The specific vector used for transformation is chosen based on the size and sequence of the foreign DNA fragment, the characteristics of the cells to be transformed, and the goal of the transformation. The goal of most genetic transformation is to express the gene product(s), and so change the traits of the organism that receives the foreign DNA. An organism with foreign DNA in its genome is said to be **transgenic**.

vector a vehicle by which foreign DNA may be introduced into a cell

transgenic a cell or an organism that is transformed by DNA from another species

plasmid a small double-stranded circular DNA molecule found in some bacteria

Transformation of Bacteria

Bacteria are the most common organisms that are transformed by molecular biologists. Transgenic bacteria may be used to study gene expression or gene function, to create and maintain a stock of a particular DNA fragment, or to synthesize a useful gene product. For example, transgenic bacteria have been engineered to produce human growth hormone, used in the treatment of pituitary dwarfism.

The first stage of transformation for any organism is to identify and isolate the DNA fragment that is to be transferred. The DNA fragment is then introduced into the vector. **Plasmids** are small, circular, double-stranded DNA molecules that occur naturally in the cytoplasm of many bacteria (**Figure 8**). Plasmids are commonly used as vectors for bacterial transformation. A plasmid contains genes, and it is replicated and expressed independently of the large bacterial chromosome. There can be many copies of a plasmid in a single bacteria cell and, under certain conditions, plasmids can pass through the cell membrane.

Figure 9, on the next page, is a diagram of the basic steps in producing transgenic bacteria. First, both the plasmid vector and the DNA containing the desired sequence are cut by the same restriction enzyme(s). In this example, both DNA molecules are cut by *Eco*RI, generating sticky ends. The cut plasmid and DNA fragment are then mixed together and incubated with DNA ligase. This produces recombinant plasmids that

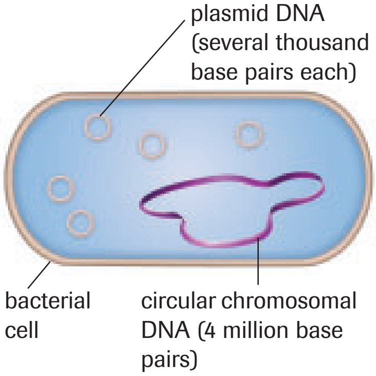


Figure 8
Chromosomal and plasmid DNA coexist in many bacteria.

EXTENSION

How to Make cDNA

One way to make copies of a particular gene is to use an enzyme called reverse transcriptase. This enzyme synthesizes DNA from mRNA. The resulting molecule is called copy DNA or cDNA. The cDNA can then be transferred into a vector or a cell.

www.science.nelson.com GO

contain the foreign DNA fragment. The bacterial cells are then treated to open pores in the cell membrane, which allows them to take up the recombinant plasmid. Once a bacterium has been transformed, it makes many copies of the recombinant plasmid, each of which includes a copy of the foreign DNA. This is often called gene cloning since the bacterium produces many identical copies (clones) of the original DNA fragment.

However, not all the bacterial cells will take up the recombinant plasmid. How can a scientist or technician distinguish between bacteria with a plasmid and those without? Plasmids used for transformation experiments often carry genes for antibiotic resistance, which can then be used to select for transformed bacteria. By growing the bacteria in medium that contains the antibiotic, any cells that do not contain a plasmid are killed off. Individual bacteria cells are then grown into colonies so that the plasmid DNA can be isolated from the cells and checked to make sure it contains the desired foreign DNA sequences.

For this transformation procedure to be successful, the plasmid DNA must have only one recognition site for the restriction enzyme that is used, or else it would be cut into a number of useless pieces. Naturally occurring plasmids do not always have a single appropriate restriction enzyme site, so scientists have engineered plasmids especially for transformation. Most of these engineered plasmids contain a **multiple-cloning site**, which is a single region that contains unique recognition sites for an assortment of restriction enzymes. The recognition sites are positioned very close together and are not found anywhere else on the plasmid's DNA sequence.

multiple-cloning site a region in a vector that is engineered to contain the recognition site of a number of restriction enzymes

Vectors other than plasmids may also be used to transform bacteria, including viruses and small inert particles that are literally fired into the cells.

DID YOU KNOW?

Plasmids: Beneficial Guests

Japanese scientists were the first to discover plasmids that carry genes for multiple drug resistance. The bacterium *Shigella*, which causes dysentery, developed resistance to as many as four antibiotics, including tetracycline, streptomycin, chloramphenicol, and the sulfonamides. The multidrug resistance was due to a plasmid within the bacterium that carried genes for resistance and could be passed naturally from bacterium to bacterium.

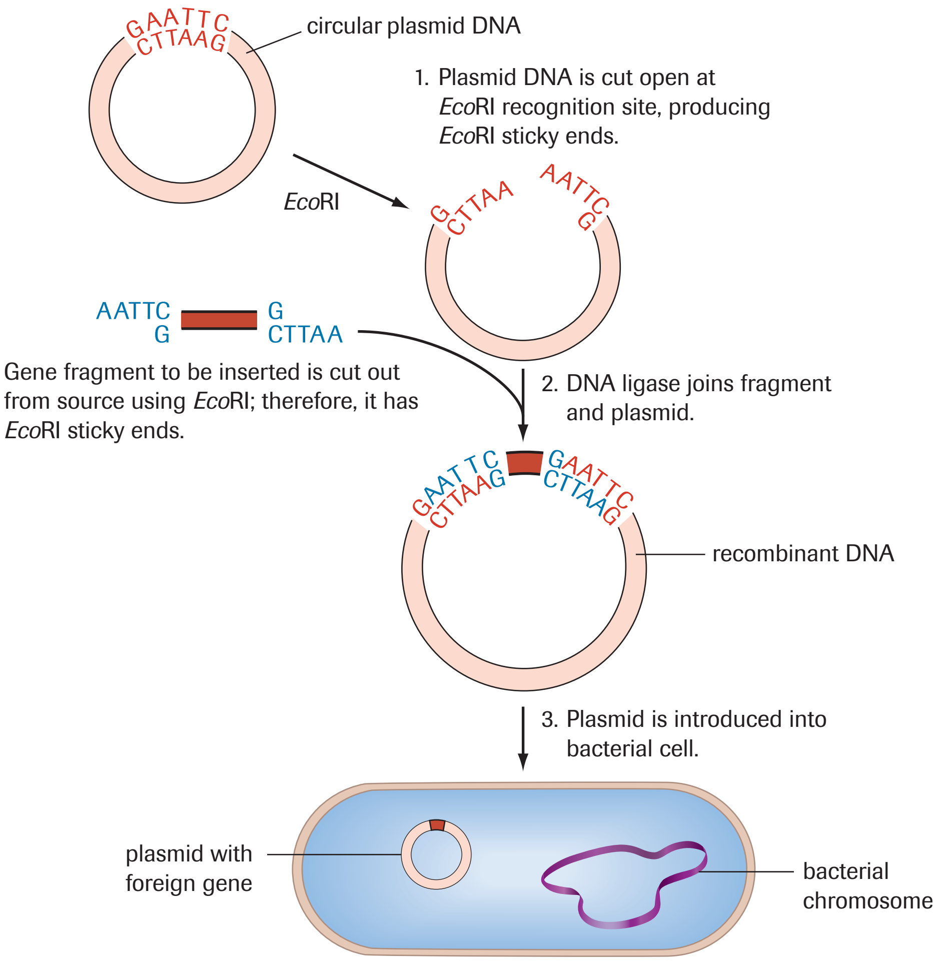


Figure 9

A foreign gene is introduced into a plasmid. The plasmid is now an example of recombinant DNA, which can be introduced into a bacterial cell to produce numerous copies of the gene.

WEB Activity

Case Study—Transformation of Eukaryotes

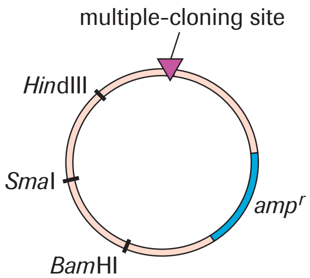
The first transgenic animal and the first transgenic plant were both produced in 1982. The animal was a mouse that contained the gene for growth hormone from a rat. The plant was a tobacco plant that contained a gene from a bacterial cell. The introduced gene produced an antibiotic in the plant's cells that protected the plant from bacterial infection. Since then, many transgenic animals and plants have been produced.

Producing transgenic eukaryotes is a lot more complex than the transformation of bacteria, and new techniques are still being developed. In this activity, you will find out about one technique used to create transgenic eukaryotes.

www.science.nelson.com GO

SUMMARY *DNA and Biotechnology*

Table 3 Key Tools of Molecular Biology

Tool	Use	Example
restriction endonuclease	bacterial enzyme that cuts DNA sequences at a specific recognition site	*Bam*HI recognition site: 5′-GGATCC-3′ 3′-CCTAGG-5′ DNA sequence before cleavage: 5′-TCAGCGGATCCCAT-3′ 3′-AGTCGCCTAGGGTA-5′ DNA sequence after cleavage with *Bam*HI: 5′-TCAGCG GATCCCAT-3′ 3′-AGTCGCCTAG GGTA-5′
methylase	enzyme that adds a methyl group to recognition sites to protect DNA from cleavage by restriction enzyme	*Bam*HI methylase adds methyl group ($-CH_3$) to second guanine nucleotide in the recognition site: 5′-GGATCC-3′ 3′-CCTAGG-5′ DNA sequence no longer cleaved by *Bam*HI methyl group changes recognition site
DNA ligase	enzyme that joins DNA fragments by creating bonds between nucleotides in the DNA backbone	DNA fragments before subjection to DNA ligase: 5′-ATAGTG-3′ 5′-AATTCGG-3′ 3′-TATCACTTAA-5′ 3′-GCC-5′ DNA fragments after subjection to DNA ligase: 5′-ATAGTGAATTCGG-3′ 3′-TATCACTTAAGCC-5′ two fragments are joined
plasmid	small circular DNA that has the ability to enter and replicate in bacterial cells and, therefore, can be used as a vector to introduce new genes into a bacterial cell	plasmid containing multiple-cloning site, ampicillin-resistance gene, and other restriction enzyme sites 

Section 20.3 Questions

1. Define *restriction endonuclease* and *methylase*.
2. Restriction endonucleases are found in many species of bacteria.
   (a) Describe their role and function in a bacterial cell.
   (b) How does the role of restriction endonucleases in nature differ from the role of restriction endonucleases in the laboratory setting?
3. Distinguish between blunt ends and sticky ends.
4. Define *recognition site*. Using examples to support your answer, depict the palindromic nature of recognition sites.
5. Restriction enzymes cut at recognition sites that are usually six to eight base pairs in length. Provide reasons why a 2-base-pair recognition site would be too short to be useful and a 14-base-pair recognition site may be too long to be useful in the field of genetic engineering.
6. Sketch a diagram that summarizes the process of polymerase chain reaction (PCR). Clearly label the important features.
7. Explain why the Human Genome Project's initial years were spent developing techniques that would sequence larger DNA strands efficiently. (*Hint:* The human genome contains approximately three billion base pairs.)
8. As a scientist working for a pharmaceutical company, you are asked to engineer bacteria that will produce human growth hormone. The objective is commercial production in order to treat individuals who are deficient in this hormone. Describe the steps you would take in order to produce this hormone.
9. Transformation technology is used in agriculture to create genetically modified organisms (GMOs) that contain useful traits. This is a controversial technology, however. Some people think that GMOs pose unacceptable environmental or health risks. The Government of Canada has set regulations that must be met for approval of GMOs. Using the Internet and other resources, research the regulations that have been put into place. Do you feel these guidelines are adequate? What modification would you make to these guidelines if you could? Explain the implications of the guidelines that have been set.

   www.science.nelson.com GO

Extension

10. In order to create recombinant DNA containing the desired sequences, scientists have developed a number of procedures to find and isolate DNA, and to confirm whether a transgenic organism contains the foreign DNA. Go to the Nelson Web site to find out how the techniques of electrophoresis, Southern blotting, and Northern blotting work and when they are used. Then, summarize the information in a chart or another appropriate format.

    www.science.nelson.com GO

11. PCR can be used to create a DNA "fingerprint" that can identify an individual. This technique has been applied to forensics. In some well-known cases, such as that of Guy Paul Morin, PCR has been used to overturn convictions made before the technology was available. In June 2000, the Government of Canada passed the DNA Identification Act, which gave the Royal Canadian Mounted Police the right to create and maintain a database of DNA fingerprints. Conduct research on the use of PCR to identify individuals. Then, use this information to prepare a convincing argument for or against the requirement that anyone accused of a serious crime must supply police with a DNA sample.

    www.science.nelson.com GO

Mutations and Genetic Variation 20.4

Mutations are changes in the sequence of the DNA molecule and are the source of new genetic variation that may be acted on by natural selection. A beneficial mutation gives an organism a selective advantage and tends to become more common over time, leading to new evolutionary changes. A harmful mutation reduces an individual's fitness and tends to be selected against. Harmful mutations occur at low rates in a species. Some mutations are neutral, having neither a benefit nor a cost, and are not acted on by natural selection.

As scientists gained more knowledge about the nature of DNA and the genetic code, they were able to more fully understand mutations. **Point mutations** are changes in a single base pair of a DNA sequence. They may or may not change the sequence of amino acids. **Gene mutations** change the amino acids specified by the DNA sequence, and they often involve more than a single base pair. **Figure 1** summarizes the DNA changes that occur in some common types of mutation.

Learning Tip

Review how mutations contribute to the variability of species and how natural selection acts on mutations in Chapter 6 of this book.

point mutation a mutation at a specific base pair

gene mutation a mutation that changes the coding for amino acids

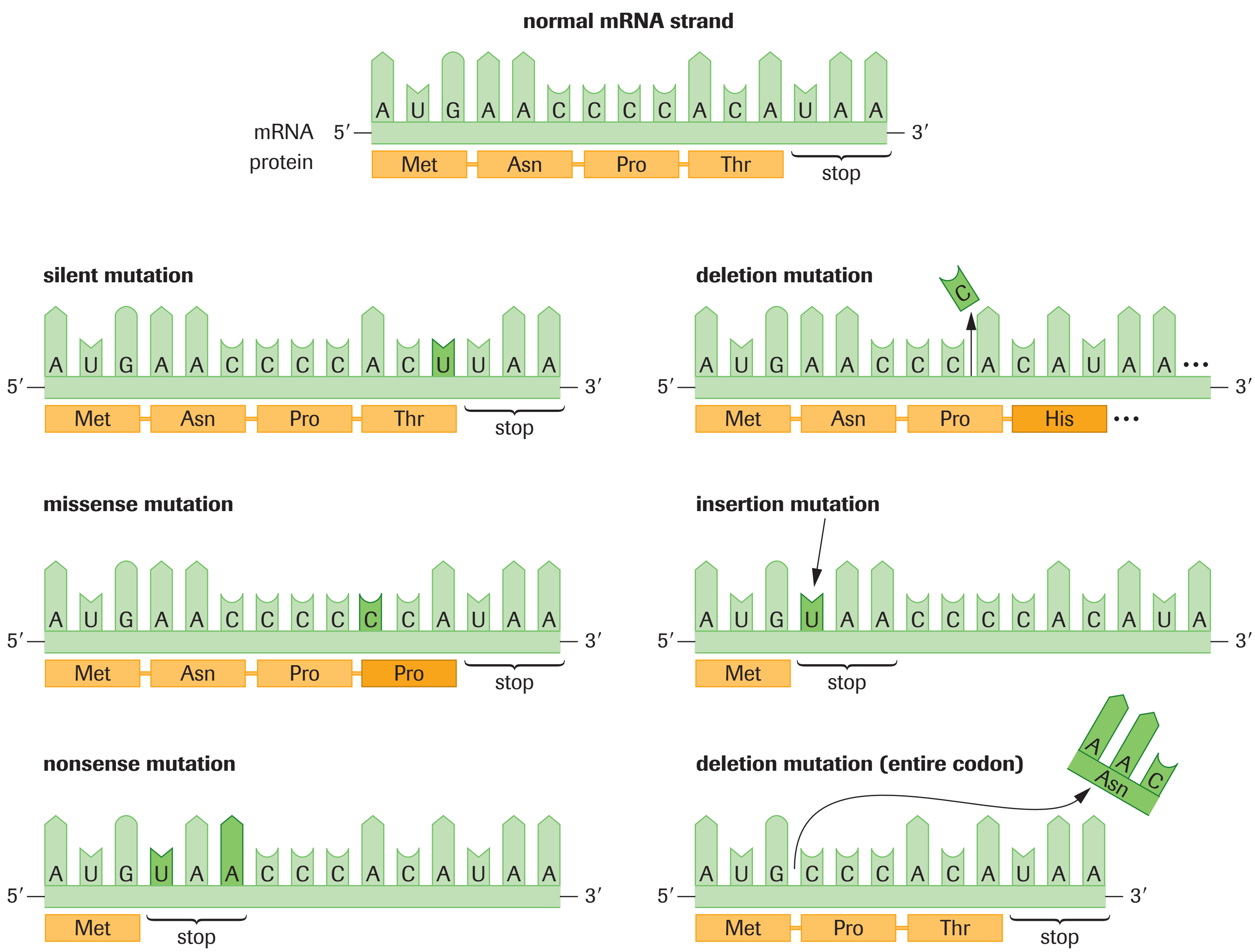


Figure 1

A summary of different types of mutations that may occur in a DNA sequence, affecting the transcribed RNA sequence.

silent mutation a mutation that does not result in a change in the amino acid coded for

missense mutation a mutation that results in the single substitution of one amino acid in the polypeptide

nonsense mutation a mutation that converts a codon for an amino acid into a stop codon

deletion the elimination of a base pair or group of base pairs from a DNA sequence

insertion the placement of an extra nucleotide in a DNA sequence

frameshift mutation a mutation that causes the reading frame of codons to change

translocation the transfer of a fragment of DNA from one site in the genome to another location

inversion the reversal of a segment of DNA within a chromosome

spontaneous mutation a mutation occurring as a result of errors made in DNA replication

mutagenic agent an agent that can cause a mutation

induced mutation a mutation caused by a chemical agent or radiation

One type of point mutation, called a **silent mutation**, has no effect on the operation of the cell. In the silent mutation example in **Figure 1**, the codon for threonine has changed from ACA to ACU. However, this mutation does not change the amino acid because both these codons code for threonine. Most silent mutations occur in the non-coding regions, so they do not affect protein structure.

A **missense mutation** arises when a change in the base sequence of DNA alters a codon, leading to a different amino acid being placed in the polypeptide. Sickle cell anemia is the result of a missense mutation. Another type of point mutation is a nonsense mutation. A **nonsense mutation** occurs when a change in the DNA sequence causes a stop codon to replace a codon specifying an amino acid. During translation, only the part of the protein that precedes the stop codon is produced, and the fragment may be digested by cell proteases. Nonsense mutations are often lethal to the cell. Missense and nonsense mutations arise from the substitution of one base pair for another.

An example of a gene mutation is a **deletion**, which occurs when one or more nucleotides are removed from the DNA sequence. In the deletion mutation example in **Figure 1**, on the previous page, a cytosine nucleotide has been deleted. This changes the third codon from CCC to CCA, but the amino acid does not change because both CCC and CCA code for proline. However, the deletion also causes a change in the fourth codon, from ACA to CAU. This does affect the amino acid, changing it from threonine to histidine. Such shifts in the reading frame usually result in significant changes to the protein.

Another way that a shift in the reading frame can occur is by the **insertion** of a nucleotide. Since the DNA sequence is read in triplets of nucleotides, inserting an extra nucleotide will cause different amino acids to be translated, similar to a deletion mutation. When a mutation changes the reading frame, it is called a **frameshift mutation**. Insertions and deletions can both cause frameshift mutations. A deletion or insertion of two nucleotides will also result in a shift of the reading frame; however, a deletion or insertion of three nucleotides does not have this effect. Instead, the insertion or deletion of three nucleotides results in the addition or removal of one amino acid.

Another category of mutations involves large segments of DNA and is seen at the chromosomal level. **Translocation** is the relocation of groups of base pairs from one part of the genome to another. Usually translocations occur between two nonhomologous chromosomes. A segment of one chromosome breaks and releases a fragment, while the same event takes place on another chromosome. The two fragments exchange places, sometimes disrupting the normal structure of genes. When unrelated gene sequences come together and are transcribed and translated, the result is a fusion protein with a completely altered function, if any. Some types of leukemia are associated with translocations and their respective fusion proteins.

Finally, an **inversion** is a section of a chrosome that has reversed its orientation in the chromosome (has turned itself around). There is no gain or loss of genetic material, but, depending on where the inversion occurs, a gene may be disrupted.

Causes of Genetic Mutations

Some mutations are simply caused by error of the genetic machinery and are known as **spontaneous mutations**. For example, DNA polymerase I occasionally misses a base or two, which results in a point mutation. Mutations may also arise from exposure to **mutagenic agents**. These are **induced mutations**. Some examples of mutagenic agents include ultraviolet (UV) radiation, cosmic rays, X-rays, and certain chemicals.

Case Study

Gene Mutations and Cancer

Cancer is considered a genetic disease because it is always associated with a mutation in the genetic sequence. However, many different things can alter DNA, including viruses and various environmental factors (**Figure 2**).

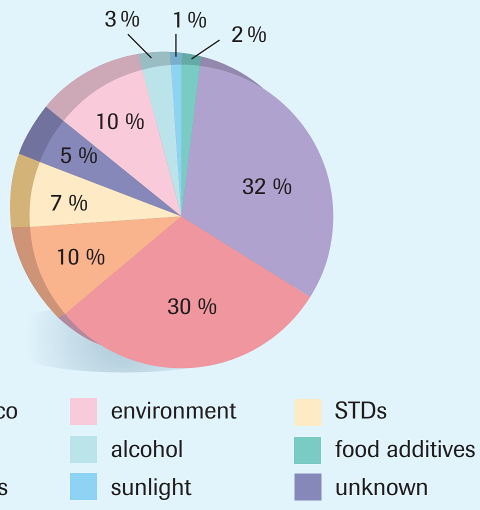


Figure 2
Estimates of risk factors for cancer calculated in percentages. Lifestyle choices related to diet and smoking can be linked with over 60 % of cancer cases.

Viruses inject foreign genetic information into cells, disrupting the DNA that codes for cell division. Some viruses that are linked to sexually transmitted diseases are known to cause cancer. For example, women who have human papillomavirus (HPV) have a greater incidence of cancer. Environmental factors have been linked to other types of cancer. Skin cancer, for example, has been linked with ultraviolet radiation from the Sun. Exposure to harmful chemicals in our environment can also cause cancer. A number of cancer-causing substances can be found in cigarettes.

Whatever the initial cause, scientists agree that all cancers are related to mutations. Usually, it takes more than one mutation to trigger a malignant growth. This is why cancer usually occurs more frequently in older people.

Two lines of evidence indicate that cancer results from mutations. First, cancer cells often display nitrogen base substitution, or the movement of genetic material from one part of the chromosome to another. Second, many known mutagens are also known to cause cancer. X-rays, ultraviolet radiation, and mutagenic chemicals can induce cancer.

In 1982, molecular biologists were able to provide additional evidence to support the hypothesis that cancer could be traced to genetic mutations. Segments of chromosomes extracted from cancerous mice were used to transform normal mouse cells (growing in tissue culture) into cancerous cells. The cancer-causing genes, called oncogenes, seemed to turn on cell division. In their noncancerous state, oncogenes are usually referred to as proto-oncogenes. Proto-oncogenes may remain inactive or may perform some useful function until they are triggered to become active oncogenes. Evidence suggests that activation occurs in a number of steps, so a single "hit" (mutation) does not immediately result in cancerous cell divisions.

Further studies indicate that cancer-causing oncogenes are present in normal strands of DNA. But if oncogenes are found in normal cells, why do normal cells not become cancerous? One current theory that has gained acceptance from the scientific community suggests that the cancer gene has been transposed (moved) to another gene site. Such transpositions may have been brought about by environmental factors or mutagenic chemicals or other agents.

Genes that direct the assembly of amino acids into proteins are referred to as structural genes. Genes called regulator genes act like a switch to turn "off" segments of the DNA molecule, so that a gene is active only when and where its gene product is needed. In very simple terms, when a mutagen causes the oncogene to become separated from its regulator gene, the cell may then be unable to turn the gene "off" (**Figure 3**). This causes the cell to continue to divide at an accelerated rate.

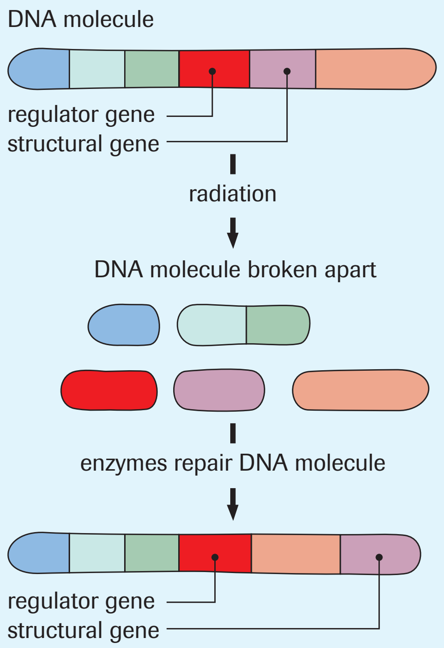


Figure 3
Mutagenic agents may cause the separation of the regulator and structural genes. If the structural gene codes for a protein involved in controlling cell division, this separation can lead to cancer.

The most common oncogene, *ras,* is found in 50 % of colon cancers and 30 % of lung cancers. Present in normal cells, *ras* makes a protein that acts as an "on" switch for cell division. *Ras* ensures that cells divide to replace damaged or dead cells. After a sufficient number of cells have been produced, the *ras* gene should be turned off. But the cancer-causing oncogene produces a protein that blocks the "off" switch. With the switch left on, cell division goes on continuously.

Case Study Questions

1. Why do many scientists believe that certain viruses cause cancer?
2. How does sunlight cause cancer?
3. List three environmental carcinogens and suggest a possible source for each.
4. Distinguish between oncogenes and proto-oncogenes.
5. Explain how oncogenes are activated.
6. What is the *ras* gene?

Inferring Relationships from DNA Sequences

At one time, scientists could compare and classify species based only on their morphology and behaviour. For example, Charles Darwin found evidence for the theory of evolution by comparing anatomical features of different species (see Chapter 6). Today, biologists can compare the genetic makeup of different species for evidence of relationships among them.

phylogeny proposed evolutionary history of a species or group of organisms

Phylogeny is the proposed evolutionary history of a group of organisms, or of a species. Overall, species that are closely related will share very similar DNA sequences, while those that are more distantly related will have more genetic differences. For example, you might expect that the sequence of DNA in a house cat's genome would have more similarities to that of a lion than to a sparrow. As we have seen, the DNA of any organism can mutate. Natural selection acts on beneficial and harmful mutations in a population, changing the relative proportions of these mutations that are passed on from generation to generation. The genomes of two species with a recent common ancestor would have had less time and opportunity for mutations to accumulate and be selected, and so we can predict that they would show fewer differences.

Learning Tip

Lab Exercise 5.A in Chapter 5 shows an example of how differences in genomic DNA sequences provide evidence for the relationships among various species.

Mutations do not occur only in genomic DNA. Nuclear DNA is often quite a large genome, so for some research it is more efficient for scientists to examine the changes in the smaller genomes of mitochondria or chloroplasts. In particular, mitochondrial DNA (mtDNA) can be used to trace inheritance through the maternal line in mammals, as the egg is the only source of the mitochondria that are passed on to new offspring.

Mitochondrial DNA has also provided some fascinating clues about the evolutionary history of modern humans. Two theories are proposed to explain the current distribution of humans around the world. One proposes that modern humans, *Homo sapiens*, evolved simultaneously in different regions of the world from an earlier species, *Homo erectus*. This theory is called the multiregional model and proposes that the different ethnic groups observed worldwide today would have begun their evolution to *Homo sapiens* between one and two million years ago. According to this model, the groups interbred to some degree, and so didn't form into different species. The second theory, called the monogenesis model, proposes that *Homo* species moved out of Africa twice: first as *Homo erectus,* and second as *Homo sapiens* between 100 000 and 200 000 years ago, and that modern ethnic groups are all descendants of the second migration.

DID YOU KNOW?

The Romanovs

Mitochondrial DNA was used to identify the suspected remains of the imperial Romanov family in Russia, who were murdered by the Bolsheviks in 1918. To do so, mitochondrial DNA from Prince Philip of England, a close relative of the former Tsarina Alexandra through his maternal side, was compared to mitochondrial DNA recovered from the remains, resulting in positive identification and the resolution of an 80-year-old mystery.

Mitochondrial DNA analyses for a variety of individuals, representing the ethnic groups found around the world, seem to support the monogenesis model. The greatest variety of mtDNA mutations exist in African ethnic groups, which is consistent with the theory that mutations accumulate over time and that the population that has existed the longest will demonstrate the largest accumulation of mutations. Additionally, the mtDNA from ethnic groups on continents other than Africa were traced back to Africa rather than to each other.

Interspersed Elements

Other DNA analyses focus on intervening sequences inserted into DNA. For example, **SINEs** (**s**hort **in**terspersed **e**lements) and **LINEs** (**l**ong **in**terspersed **e**lements) are often associated with the genes of retroviruses within the genome and are thought to have been inserted by those viruses. SINEs and LINEs are often located in areas of the DNA that appear to be noncoding regions. That is, the DNA in these areas does not code for one of the known gene products of that species. Although the function of the DNA in these regions is not known, it is inherited; therefore, changes to these DNA sequences, such as insertions, are passed to succeeding generations.

If two species have the same SINE or LINE located at precisely the same position in their DNA, it can be assumed that the insertion occurred only once in a common ancestor. SINEs and LINEs make ideal markers for tracing evolutionary pathways. They are easy to find and identify, even if they undergo small mutational changes, because they are relatively large and recognizable segments of DNA often hundreds of base pairs in length. The possibility of a mutation reverting to an older form is extremely remote, as the chances of a SINE or LINE being inserted in exactly the same location in two different species is highly unlikely.

SINEs repeated DNA sequences 300 base pairs long that alternate with lengths of DNA sequences found in the genomes of higher organisms

LINEs repeated DNA sequences 5000 to 7000 base pairs long that alternate with lengths of DNA sequences found in the genomes of higher organisms

LAB EXERCISE 20.B

Looking for SINEs of Evolution

Report Checklist

○ Purpose	○ Design	● Analysis
○ Problem	○ Materials	● Evaluation
○ Hypothesis	○ Procedure	● Synthesis
○ Prediction	○ Evidence	

In this activity, you will use DNA sequences to predict and chart phylogenetic relationships among species.

Suppose you find a pattern in the noncoding SINE DNA of two different species, and do not find that pattern in other species. Evolution can explain the situation by saying that the two species recently had a common ancestor, and that both species inherited this pattern from their ancestor. The predicted family tree is shown in **Figure 4**.

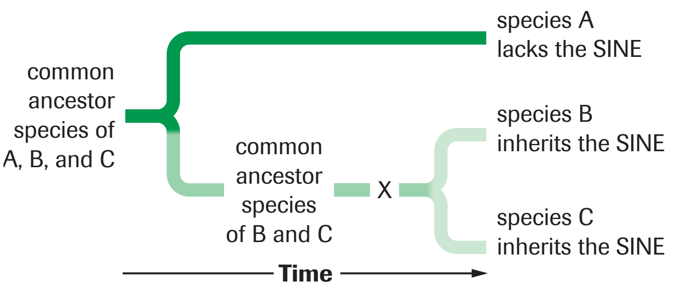


Figure 4
X indicates the time when the SINE became inserted into the genome. Since the SINE insertion occurs only once, at time X, the size and precise location of the SINE will be identical in species B and C.

Part I: Looking for a SINE

Procedure I

1. Examine the hypothetical DNA code from four different species (**Figure 5**). These species have large sections of DNA that appear to be homologous. These homologous sequences have been aligned vertically so that similarities and differences can be easily seen and colours are used to highlight those nucleotides that are not matches (**Figure 6**).

```
Species W   AGATAGCGCGTAAAAAG
Species X   AAATAGCGCGTAAATAG
Species Y   AAATAGTTAAAGTTACGCATAAATAC
Species Z   AGATAGCGCGTAAATGG
```

Figure 5
Sequenced DNA fragments from four distantly related species

```
Species W-   AGATAG.........CGCGTAAAAAG
Species X-   AAATAG.........CGCGTAAATAG
Species Y-   AAATAGTTAAAGTTACGCATAAATAC
Species Z-   AGATAG.........CGCGTAAATGG
```

Figure 6
DNA sequences from **Figure 5** aligned for comparison. Note that spaces appear in the sequences only to facilitate comparisons.

LAB EXERCISE 20.B *continued*

The single nucleotide differences have most likely resulted from point mutations, while the nine-nucleotide segment in species Y is probably the result of an insertion. (Note that this is much more likely than the alternative possibility—that each of the other species experienced an identical deletion event in its past.) The type of pattern observed in species Y often results from a SINE or LINE insertion.

2. Copy the DNA sequences in **Figure 7** into your notebook. Align the homologous sections vertically.
3. Use a highlighter to colour all positions that have the same nucleotide in all four species.
4. Use a different colour to highlight the SINE insertion.

Analysis and Evaluation I

(a) Identify any nucleotide differences in the SINE sequences. Explain how these differences might have occurred.

(b) Identify whether mutations that occur within the SINE are likely to be harmful, beneficial, or neutral. Explain.

(c) Based on the data alone, construct a chart similar to **Table 1** showing the phylogenetic relationship of these species.

Part II: Evolution Displayed by SINEs and LINEs

Procedure II

5. Study the data in **Table 1**. DNA sequencing was used to document the presence or absence of interspersed elements A through I in five mammals. Camels are included as the outgroup.

Table 1 Molecular Evidence for the Evolution of Whales*

Group	SINE or LINE								
	A	B	C	D	E	F	G	H	I
cow	+	+	–	–	–	–	+	–	+
pig	–	–	–	+	+	–	–	–	+
whale	–	+	+	–	–	–	+	+	+
deer	+	+	–	–	–	–	+	–	+
hippopotamus	–	+	+	–	–	+	+	+	+
camel	–	–	–	–	–	–	–	–	–

\+ indicates presence of element   - indicates absence of element
* Data modified from Nikaido 1999

6. Use the data to construct a chart showing the phylogenetic relationships between these mammals. Clearly indicate the relative positions at which each insertion most likely occurred.

Analysis and Evaluation II

(d) Are whales more closely related to cows or hippopotamuses? Explain your rationale.

(e) Identify which insertion happened first: A or B? Explain your reasoning.

Synthesis

(f) Explain whether pigs and camels are more closely related than hippopotamuses and camels.

(g) What must be true about the genomes of all whale species (i.e., which SINEs must they all contain)? Explain your rationale.

(h) A researcher interested in the evolution of whales wants to know whether orcas are more closely related to white-sided dolphins or to pilot whales. Describe a way to answer this question.

```
Species P  ...AAATTGCTTCGTATTTTCGAATTGCCCCGCTAAAGCGCTTTAGC.......
Species Q  ...AACTTGCTTCGTATTAAGCTGTTGCGTAAAGTTAGTACGAATTGCCCCGGTGAAGCGCTTTAGC......
Species R  ...AATTTGCTTCGTTTTTTCGAATTGCCCCGCTAAAGCGCTTTAGC.......
Species S  ...AACTTGCTACGTATTAAGCCGTTGCGTAAAGTTAGGACGAATCGCCACGGTGACGCGCTTGAGC......
```

Figure 7
Homologous DNA sequences from four species

SUMMARY Mutations and Genetic Variation

Table 2 Types of Mutations

Category	Type	Result
point mutation	**substitution** AAG CCC GGC AAA AAG ACC GGC AAA	**missense mutation** only one amino acid substituted
	deletion AAG CCC GGC AAA AAC CCG GCA AA	**frameshift mutation** can result in many different amino acids substituted or a stop codon read (nonsense mutation)
	insertion AAG CCC GGC AAA AAG ACC GGG CAA A	
chromosomal	**translocation** chromosome 1 5′ AAATTCG GCACCA 3′ chromosome 2 5′ TAGCCC AAGCGAG 3′ ↓ chromosome 1 5′ TAGCCC GCACCA 3′ chromosome 2 5′ AAATTCG AGCGAG 3′	inactivation of gene if translocation or inversion is within a coding segment
	inversion normal chromosome 5′ AATTGGCCATA ATATGAA AAGCCC 3′ 3′ TTAACCGGTAT TATACTT TTCGGG 5′ ↓ after inversion 5′ AATTGGCCATA TTCATAT AAGCCC 3′ 3′ TTAACCGGTAT AAGTATA TTCGGG 5′	

- In mammals, mitochondrial DNA can be used to trace inheritance through the maternal lineage.
- Comparisons of DNA sequences can provide detailed phylogenetic relationships by revealing the specific changes in the genetic makeup of species and populations.
- SINEs and LINEs provide excellent inheritable markers for tracing the evolution of species' lineages.

Section 20.4 Questions

1. Clearly define the following terms and give an example of each: *mutation, frameshift mutation, point mutation, nonsense mutation, missense mutation*.
2. Explain why mutations, such as insertions or deletions, are often much more harmful than nitrogen-base substitutions.
3. Which of two types of mutations, nonsense or missense, would be more harmful to an organism? Explain your answer using your knowledge of protein synthesis.
4. Identify three factors that can produce gene mutations.
5. Identify the type of mutation that has occurred in the strands below. Describe the effect on the protein. The original strand is

   AUG UUU UUG CCU UAU CAU CGU

   Determine whether or not the following mutations would be harmful to an organism. Translate the mRNA sequence into protein to help you decide. The mutation is indicated in red.

   (a) AUG UUU UUG CCU UAU CAU CGU
   AUG UUU UUG CCU UAC CAU CGU

   (b) AUG UUU UUG CCU UAU CAU CGU
   AUG UUU UUG CCU UAA CAU CGU

   (c) AUG UUU UUG CCU UAU CAU CGU
   AUG UUU CUU GCC UUA UCA UCG U

   (d) AUG UUU UUG CCU UAU CAU CGU
   AUG UUU UUG CCU AUC AUC GU

   (e) AUG UUU UUG CCU UAU CAU CGU
   UGC UAC UAU UCC GUU UUU GUA
6. Which of the following amino acid changes can result from a single base-pair substitution?
   (a) arg to leu
   (b) cys to glu
   (c) ser to thr
   (d) ile to ser
7. Explain why a food dye that has been identified as a chemical mutagen poses greater dangers for a developing fetus than for an adult.
8. List three changes that can be made to your personal lifestyle that would reduce the odds of a mutation taking place.
9. Explain how mutations may be of benefit to an organism, and describe how these beneficial mutations are maintained in a species. Identify the biological process that influences which mutations stay in a population over time.
10. Both mitochondria and chloroplasts contain their own genomes, which are separate from the nuclear genome. The DNA in mitochondria and chloroplasts have been used as evidence for the endosymbiotic theory of the evolution of eukaryotic organisms. This theory was developed by the American scientist Dr. Lynn Margulis. According to this theory, mitochondria and chloroplast arose from bacteria and algae cells that became engulfed by another cell with which they had a symbiotic relationship. Over time, the bacteria and algae became a part of the other cell. Evidence of this theory can be found by comparing the DNA of mitochondria with bacteria, and of chloroplasts with algae. Go to the Nelson Web site to learn more about the theory of endosymbiosis, and summarize the DNA evidence that supports it.

    www.science.nelson.com GO

Extension

11. The mutation that causes sickle cell anemia involves the substitution of the amino acid valine for the amino acid glutamic acid. Research the structure of valine and glutamic acid and, with your knowledge of chemistry, hypothesize why this substitution results in a large conformational change for the hemoglobin protein. List other amino acids that could have been substituted instead of valine that may not have caused such serious side effects. List amino acids that are similar to glutamic acid that would probably cause similar side effects.

INVESTIGATION 20.1

Report Checklist

- ● Purpose
- ○ Problem
- ● Hypothesis
- ● Prediction
- ● Design
- ○ Materials
- ○ Procedure
- ● Evidence
- ● Analysis
- ● Evaluation
- ● Synthesis

Protein Synthesis and Inactivation of Antibiotics

In this investigation, you will examine the effects of ampicillin on two types of bacteria. *E. coli* MM294/pAmp contains a gene insert that directs the synthesis of a protein that inactivates ampicillin, whereas *E. coli* MM294 does not. Ampicillin inhibits bacterial growth by interfering with cell wall biosynthesis. Based on your knowledge of protein synthesis, make a prediction about the survival of *E. coli* MM294/pAmp and *E. coli* MM294 on ampicillin-rich media.

Problem

What effect does the presence of an ampicillin-resistance gene in a bacterium have on its growth on ampicillin-rich media?

Materials

apron	masking tape
safety goggles	permanent marker
gloves	inoculating loop
10 % bleach	Bunsen burner
2 LB agar plates	MM294 culture
2 LB + ampicillin (LB/amp) plates	MM294/pAMP culture
	37 °C incubator

Wear safety goggles at all times.

Wear gloves when performing the experiment. Disposable latex gloves are best avoided since allergic reactions to latex have been widely reported. Disposable polyethylene, PVC, or neoprene gloves are recommended.

Wipe down all surfaces with 10 % bleach before and after the laboratory exercise.

All resulting cultures must be immersed in 10 % bleach before disposal to ensure sterilization.

Do not leave a lit Bunsen burner unattended. Refer to Appendix C2 for a review of the safe use of a Bunsen burner.

Wash your hands thoroughly at the end of the laboratory.

Procedure

1. Put on your safety goggles and gloves, and wipe down your bench with a 10 % bleach solution.
2. Obtain two LB plates and two LB/amp plates from your teacher.
3. Label the bottom of each plate with your name and the date, using a permanent marker.
4. Label both of the LB plates "− amp" for the *E. coli* MM294 cells. Label both of the LB/amp plates "+ amp" for the *E. coli* MM294/pAMP cells.
5. Hold your inoculating loop like a pencil and sterilize it in the nonluminous flame of the Bunsen burner until it becomes red hot. Cool the sterilized loop by touching it to the edge of the agar on one of the LB plates.
6. Using the sterilized loop, pick up one colony of *E. coli* MM294 from a start culture plate. Glide the inoculating loop across an LB agar plate, making sure not to gouge the agar (**Figure 1**).

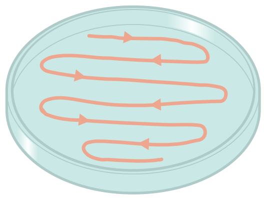


Figure 1
Pattern of streaking on an agar plate

7. Resterilize your loop as directed in step 5.
8. Repeat step 6 with *E. coli* MM294 streaked on an LB/amp plate.
9. Resterilize your loop as directed in step 5.
10. Repeat step 6 with *E. coli* MM294/pAmp streaked on the other LB plate.
11. Resterilize your loop as directed in step 5.
12. Repeat step 6 with *E. coli* MM294/pAmp streaked on the other LB/amp plate.
13. Sterilize and cool your inoculating loop.
14. Place all four streaked plates in a stack and tape them together. Seal the edges of your plates with masking tape.
15. Place the streaked plates upside down in the incubator. Alternatively, if you do not have an incubator, place the plates in a warm part of the room for a couple of days.

INVESTIGATION 20.1 *continued*

16. Disinfect your laboratory bench using the bleach solution.
17. Wash your hands thoroughly with soap and water.

Analysis

(a) After sufficient time has elapsed, remove your plates from the incubator and note any changes.

Never open the plates, as any bacterial colonies within are a potential source of contamination. If condensation has accumulated on one side of a plate, try looking through its bottom to observe the colonies you may have cultured. Once the experiment has been completed, flood plates with bleach to kill the bacterial colonies that have been cultured. Alternatively, place plates in an autoclave before they are disposed.

Evaluation

(b) Compare your results to your prediction. Explain any possible causes for variation.

(c) What evidence is there to indicate that protein was synthesized by the bacteria?

(d) Why was it important to streak out both types of bacteria on both types of plates?

(e) This experiment contains both positive and negative controls. Identify them. What information do the controls provide in this experiment?

(f) Why was it important to cool the inoculating loop before obtaining a bacterial colony from a stock plate?

(g) Why was it important to resterilize the inoculating loop between transfers of bacteria?

(h) Suggest possible sources of error in this procedure and indicate their effect on the results.

Synthesis

(i) *E. coli* strains containing the genetic sequence pAmp are resistant to ampicillin. Research how the ampicillin can be deactivated by β-lactamase, the protein coded for by the ampicillin-resistance gene.

(j) Predict what would happen if there was an error in the genetic sequence that codes for β-lactamase.

INVESTIGATION 20.2

Restriction Enzyme Digestion of Bacteriophage DNA

In this investigation, bacteriophage lambda DNA will be digested using the restriction endonucleases *Eco*RI, *Hin*dIII, and *Bam*HI. The fragments produced will be separated using gel electrophoresis. Fragment sizes will be calculated from an analysis of the agarose gel. Bacteriophage lambda DNA is obtained from a virus that infects bacterial cells and is 48 514 base pairs in length.

Before you begin, predict the number and size of the DNA fragments you will obtain, using the restriction enzyme site map shown in **Figure 1** on the next page.

Report Checklist

● Purpose	● Design	● Analysis
○ Problem	○ Materials	● Evaluation
● Hypothesis	○ Procedure	○ Synthesis
● Prediction	● Evidence	

Problem

How do the patterns of DNA fragments compare when a piece of DNA is digested using different restriction endonucleases?

Materials

safety goggles
gloves
70 % ethanol solution (or 10 % bleach)
4 1.5 mL Eppendorf tubes
waterproof pen for labelling
masking tape
polystyrene cup
freezer
crushed ice
20 μL of 0.5 μg/μL lambda DNA
5 μL 10× restriction buffer
1.0–20 μL micropipette with tips
2 μL each of *Bam*HI, *Eco*RI, and *Hin*dIII restriction endonucleases

INVESTIGATION 20.2 *continued*

microcentrifuge (optional)
37 °C water bath
thermometer
1 g agarose
paper boat
electronic balance
500 mL Erlenmeyer flask
250 mL graduated cylinder
microwave or hot plate
flask tongs or oven mitts
gel casting tray and gel electrophoresis box
1L 1× TBE buffer
5 μL loading dye
power supply (45 V)
plastic wrap
25–30 mL 0.025 % methylene blue, or enough to cover the gel in the staining tray
light box or overhead projector
acetate sheet

Wear safety goggles at all times.

Wear gloves when performing the experiment.

Wipe down all surfaces with 70 % ethanol, or 10 % bleach, before and after the laboratory exercise.

Do not use ethanol near a heat source.

Wash your hands thoroughly at the end of the laboratory.

Procedure

Day 1: Restriction Enzyme Digestion

1. Put on your safety goggles and gloves, and wipe down your bench with a 70 % ethanol solution (or 10 % bleach).

Ethanol is highly flammable. Make sure that any flame on your desk or near it is turned off before use.

2. Label four 1.5 mL Eppendorf tubes "*Bam*HI," "*Eco*RI," "*Hin*dIII," and "control." Place the tubes in a polystyrene cup containing crushed ice. **Table 1** outlines the amount of reagents to add to each tube. To keep track of each tube's contents, copy the table into your notebook and check off each reagent as you add it to the tube.

Table 1 Reagents to Add to Tubes

Tube	DNA (μL)	10× buffer (μL)	Water (μL)	*Bam*HI (μL)	*Eco*RI (μL)	*Hin*dIII (μL)
***Bam*HI**	4	1	4	1	–	–
***Eco*RI**	4	1	4	–	1	–
***Hin*dIII**	4	1	4	–	–	1
control	4	1	5	–	–	–

3. Read down each column, adding the same reagent to all appropriate tubes. Use a fresh tip on the micropipette for each reagent. Add the 4 μL of DNA to each tube first, followed by the 10× reaction buffer, and then the water. *Make sure you add the enzyme last.* Dispense all the contents close to the bottom of the Eppendorf tubes. Ensure that the pipette tip is touching the side of the tubes when dispensing the contents. *Keep everything on ice at all times.*
4. Close the Eppendorf tube tops. Place the tubes in the microcentrifuge, close it, and spin at maximum speed for approximately 3 s. If you do not have access to a microcentrifuge, then just tap the tubes on a soft pad or thick paper towel on the bench, pooling the contents to the bottom.

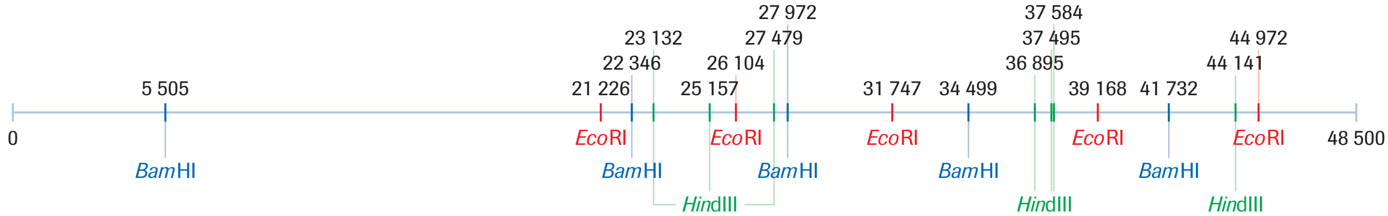


Figure 1
Restriction enzyme map of bacteriophage lambda DNA

INVESTIGATION 20.2 *continued*

When using the microcentrifuge:

- **Do not open the centrifuge until it stops completely.**
- **If the centrifuge tubes are smaller than the metal holder or holes, use the proper adaptor to accommodate them.**
- **Do not unplug the centrifuge by pulling on the cord. Pull the plug.**

5. Place the tubes in a 37 °C water bath for a minimum of 45 min. Use a thermometer to check the temperature of the water.
6. Once the digestion is complete, place the tubes in the polystyrene cup and put the cup in a freezer until your next class. Make sure you have labelled your cup with your name.

Day 2: Gel Electrophoresis

7. Measure 0.96 g of agarose powder in a paper boat on an electronic balance and transfer to a 500 mL Erlenmeyer flask.
8. Use a graduated cylinder to add 125 mL of 1× TBE buffer and swirl to mix.
9. Heat the flask on a hot plate or in a microwave until the solution is completely clear. Handle carefully, using tongs or oven mitts. Make sure you wear goggles and a lab coat.

If the agarose gets too hot it may bubble over. Be sure to observe your Erlenmeyer flask throughout the heating process. If the agarose solution starts to bubble up the neck of the flask, remove it immediately from the heat source using an oven mitt or tongs. Handle all hot glassware with caution.

10. Prepare the gel casting tray. Depending on your gel electrophoresis unit, you may have to tape the gel casting tray. Ensure that the plastic comb is inserted properly.
11. Once the flask with agarose solution is cool enough to handle with bare hands, pour the mixture into the gel casting tray. The comb teeth should be immersed in about 6 mm of agarose. The gel should cover only about one-third of the height of the comb teeth. Use a micropipette tip to remove bubbles from the gel as soon as it is poured.
12. Allow the agarose to set for a minimum of 20 min. The gel will become cloudy as it solidifies.
13. Once the gel has set (you may test this by gently touching the lower righthand corner with your finger), flood the gel with 1× TBE running buffer and then pull out the comb gently without ripping any of the wells.
14. Orient the tray containing the gel in the gel electrophoresis box so that the wells made by the comb are at the end with the positive electrode.
15. Add 1× TBE buffer to the gel electrophoresis box until the buffer is approximately 5 mm above the gel. Place the gel electrophoresis box to the side.
16. Add 1 μL of loading dye to each of the Eppendorf tubes. Microfuge for 3 s.
17. Micropipette the full contents of one Eppendorf tube into a well on the gel. Do the same for each tube. Be sure to record the order in which you dispense the tubes. Steady the micropipette over each well using both hands.
18. Close the gel box and connect it to the power supply. If you are using a gel box that you made, set the voltage to 45 V dc and turn it on. Electrophorese for 12 h. Alternatively, if you have a stronger power supply or a store-bought electrophoresis unit, electrophorese at 110 V for 2.5 h.

When using the power supply:

- **Be sure the grounding pin in the power supply is not broken.**
- **Pull the plug, not the cord, when unplugging the power source.**
- **Do not let the wire leads connected to the electric power supply or batteries touch each other.**

19. Unplug the power supply and carefully remove the gel. Wrap the gel in plastic wrap and place it in the refrigerator for a maximum of one day.

Day 3: Staining the Gel

20. Unwrap the gel and place it in the staining tray.
21. Flood the gel with 0.025 % methylene blue solution. Let the gel sit in the solution for at least 20 to 25 min. Pour off the water and replace it with fresh water. Repeat this process three more times. Keep an eye on the intensity of the DNA bands. If you destain for too long, you may lose the smaller fragments.

INVESTIGATION 20.2 *continued*

If you do not destain for long enough, the whole gel remains blue and the fragments cannot be differentiated.

22. Place the destained gel on a light box or on an overhead projector.
23. Obtain a blank acetate sheet or plastic wrap and place it over the gel. Trace the pattern of bands onto the wrap or sheet. Be sure to draw a line where the bottom of each well starts.

Evidence

(a) Carefully measure the distance in millimetres that each band migrated from the well origin. Copy **Table 2** into your notebook and use it to record the distances.

Analysis

(b) Using the *Hin*dIII digestion as a marker, plot the distance travelled (*x*-axis) versus the fragment base-pair size (*y*-axis) on semilogarithmic paper. Please note that the 23 130-base-pair fragment and the 27 491-base-pair fragment do not resolve, but instead travel as one band. Therefore, take an average of their size for graphing purposes.

(c) Using interpolation, determine the fragment size of the bands produced by digestion with *Bam*HI and *Eco*RI. Enter your calculated base-pair fragment sizes into your table.

(d) Compare the calculated base-pair fragments to the actual base-pair fragments. Use the restriction enzyme map of bacteriophage lambda (**Figure 1**) to determine the size of the actual band fragments for each enzyme. Calculate the percentage error.

Evaluation

(e) What was the purpose of each tube? of the control?

(f) Why do the smaller bands migrate faster than the larger bands?

(g) Some bands that are close in size migrate together. What measures may be taken to resolve bands close in size?

(h) What purpose does the 1× running buffer serve?

(i) Why must the gel be made using 1× TBE buffer?

(j) During electrophoresis, bubbles are produced at the anode and at the cathode. Explain why bubbles appear.

(k) Why must loading dye be added to the samples before they are loaded into the wells of the gel?

(l) Notice on your gel that the larger fragments are stained darker than the smaller fragments. Explain why this is the case.

(m) Suggest possible sources of error in this procedure. Indicate the effects of these sources of error on the results.

Table 2 Distance Travelled by Each Band From the Well Origin

***Hin*dIII**		***Eco*RI**			***Bam*HI**		
Actual fragment size	**Distance travelled (mm)**	**Actual fragment size**	**Distance travelled (mm)**	**Calculated fragment size**	**Actual fragment size**	**Distance travelled (mm)**	**Calculated fragment size**
27 491							
23 130							
9 416							
6 557							
4 361							
2 322							
2 027							

Chapter 20 SUMMARY

Outcomes

Knowledge

- describe, in general, how genetic information is contained in the sequence of bases in DNA molecules in chromosomes; how the DNA molecules replicate themselves; and how the genetic information is transcribed into sequences of bases in RNA molecules and is finally translated into sequences of amino acids in proteins (20.1, 20.2)
- explain, in general, how restriction enzymes cut DNA molecules into smaller fragments and how ligases reassemble them (20.3)
- explain, in general, how cells may be transformed by inserting new DNA sequences into their genomes (20.3)
- explain how a random change (mutation) in the sequence of bases results in abnormalities or provides a source of genetic variability (20.4)
- explain how sequences of nucleic acids contained in the nucleus, mitochondria, and chloroplasts gives evidence for the relationships among organisms of different species by examining similarities and differences in base sequences (20.4)

STS

- explain that science and technology have both intended and unintended consequences for humans and the environment (20.3, 20.4)
- explain that scientific research and technological development help achieve a sustainable society, economy, and environment (20.3, 20.4)

Skills

- ask questions and plan investigations (20.4)
- conduct investigations and gather and record data and information (20.2, 20.3, 20.4)
- analyze data and apply mathematical and conceptual models to develop and assess possible solutions (20.2, 20.4)
- work as members of a team and apply the skills and conventions of science (all)

Key Terms

20.1

complementary base pairing
antiparallel
DNA replication
semiconservative replication
template
DNA helicase
DNA polymerase III
leading strand
lagging strand
DNA polymerase I
DNA ligase

20.2

gene expression
ribonucleic acid (RNA)
transcription
messenger RNA (mRNA)
translation
RNA polymerase
promoter
template strand
termination sequence
codon
start codon
stop codon
ribosome
transfer RNA (tRNA)
anticodon

20.3

recombinant DNA
genetic transformation
restriction endonuclease
recognition site
palindromic
sticky ends
blunt ends
methylase
polymerase chain reaction (PCR)
vector
transgenic
plasmid
multiple-cloning site

20.4

point mutation
gene mutation
silent mutation
missense mutation
nonsense mutation
deletion
insertion
frameshift mutation
translocation
inversion
spontaneous mutation
mutagenic agent
induced mutation
phylogeny
SINEs
LINEs

MAKE a summary

1. Starting with the title “The Human Genome,” produce a flowchart that illustrates the flow of information from gene to protein. Include as many key concepts as possible.
2. Revisit your answers to the Starting Points questions at the beginning of the chapter. Would you answer the questions differently now? Why?

Go To

www.science.nelson.com GO

The following components are available on the Nelson Web site. Follow the links for *Nelson Biology Alberta 20–30*.

- an interactive Self Quiz for Chapter 20
- additional Diploma Exam-style Review Questions
- Illustrated Glossary
- additional IB-related material

There is more information on the Web site wherever you see the Go icon in the chapter.

EXTENSION

CBC radio ONE QUIRKS & QUARKS

DNA Motors

Dr. Vanessa Auld, Quirks and Quarks genetics columnist explains the details behind the discovery by a group of American and Czech researchers of proteins that act like small motors inside the nucleus of the cell. This discovery is changing our understanding of how DNA is used to manufacture the proteins and chemicals the cell uses to sustain life.

www.science.nelson.com GO

EXTENSION

Cracking the Code of Life

In this *NOVA* video, follow corporate and academic scientists as they race to capture one of the biggest prizes in scientific history: the complete, letter-by-letter sequence of genetic information that defines human life—the human genome.

www.science.nelson.com GO

EXTENSION

Artificial Life

Scientists can now synthesize strands of DNA with any nucleotide sequence they want. Does this mean that they can create artificial life from these blueprints? Some scientists believe the answer is yes, and that it isn't that far away! Find out why by watching this *NOVA* video.

www.science.nelson.com GO

EXTENSION

Golden Rice or Frankenfood?

Vitamin A deficiency is a leading cause of preventable blindness. Scientists have developed a genetically-modified rice that contains β-carotene, the precursor to vitamin A. Some see this new rice as an important contribution to world health, but others warn that genetically modified foods could have hidden dangers. What do you think?

www.science.nelson.com GO

UNIT 30 C PERFORMANCE TASK

Investigating Human Traits

In this Performance Task, you will use the skills you gained in this Unit to design and carry out a correlational study on human traits to determine if they are autosomal or sex-linked. Go to the Unit 30 C Performance Task link on the Nelson web site to complete the task.

www.science.nelson.com GO

Chapter 20 REVIEW

Many of these questions are in the style of the Diploma Exam. You will find guidance for writing Diploma Exams in Appendix A5. Science Directing Words used in Diploma Exams are in bold type. Exam study tips and test-taking suggestions are on the Nelson Web site.

www.science.nelson.com GO

DO NOT WRITE IN THIS TEXTBOOK.

Part 1

Use the following information to answer questions 1 to 3.

The cause of cystic fibrosis has been identified as a variety of mutations to the *CFTR* gene on chromosome 7. The most common of these involves the loss of three nucleotides, which in turn results in the loss of a phenylalanine at amino acid position 508.

1. Identify the DNA sequence that would result in phenylalanine being placed in a polypeptide chain.
  A. UUG
  B. AAC
  C. UUU
  D. TTT

2. Identify the term that best describes the mutation that causes the loss of phenylalanine.
  A. silent mutation
  B. insertion mutation
  C. deletion mutation
  D. missense mutation

3. NR Gene therapy trials to correct this defect in the *CFTR* gene have been conducted by doctors in several centres. The following is a list of some genetic technologies that might be used in this work:
  1. restriction endonucelases
  2. mtDNA
  3. polymerase chain reaction
  4. DNA ligase
  5. viruses
  6. bacterial plasmids
  7. gene sequencing

  Identify the technologies that would most likely be used to isolate the gene for a therapy trial. (Record all four digits of your answer in the order in which the technologies would be used.)

4. Identify the enzyme that is correctly matched with its function.
  A. DNA polymerase I: synthesis of the continuous matching strand
  B. DNA helicase: synthesis of messenger RNA
  C. DNA polymerase III: cuts out the primer and replaces it with DNA nucleotides
  D. DNA ligase: links adjacent nucleotides together by covalent bond

5. Select the response that correctly identifies the complementary DNA strand for this strand:
5′-TACTTTGGCCCAGAG-3′
  A. 3′-AUGAAACCGGGUCUC-5′
  B. 3′-UACUUUGGCCCAGA-5′
  C. 3′-ATGAAACCGGGTCTC-5′
  D. 5′-ATGAAACCGGGTCTC-3′

Use the following information to answer questions 6 and 7.

1. Amino acids are brought to the ribosome and linked together in the correct order.
2. A copy of the gene is taken to the ribosome.
3. RNA polymerase attaches to the promoter site.
4. The two subunits of the ribosome attach to the RNA strand.
5. DNA polymerase III makes a matching strand using complementary base pairs.
6. Release factor binds to the A site and the ribosome releases the amino acid chain.
7. The two original strands serve as templates for the synthesis of new matching stands.
8. The lagging strand is synthesized in short fragments.
9. The two strands are unwound and the hydrogen bonds are broken.

6. NR Identify the steps described above that correspond to the process of replication. (Record all four digits of your answer in the order the steps would occur in the cell.)

7. NR Match these terms to the selection above that best describes them. (Record all four digits of your answer.)

________	________	________	________
initiation of transcription	termination of translation	elongation of amino acid chain	initiation of translation

Part 2

8. Use a diagram to **illustrate** how the two DNA strands in a double helix run antiparallel. Make sure you label your diagram.
9. **How** does the fact that DNA replicates semiconservatively decrease the possibility of errors made during DNA replication? **Describe** another mechanism that minimizes DNA replication error.
10. Numerous enzymes are involved in DNA replication. **Outline** the role that the following enzymes play: DNA ligase, DNA gyrase, DNA helicase, DNA polymerase I, and DNA polymerase III.
11. What is the complementary strand of AATTGCATA?
12. DNA polymerase III can only extend an existing DNA strand in the 5′ to 3′ direction. **Describe** the mechanisms in place that compensate for DNA polymerase III's inability to intitiate a strand and for its stringent directionality.
13. One strand of a DNA molecule contains the nucleotide proportions 15 % adenine (A), 30 % thymine (T), 20 % guanine (G), and 35 % cytosine (C). **Predict** the proportions of the four base pairs in the double-stranded form of this DNA.
14. **Describe** the function of mRNA and tRNA in protein synthesis.
15. **Distinguish** between transcription and translation. Use a table to organize your answer.
16. The following is a sequence of DNA for a hypothetical peptide:
    5′- AAGTACAGCAT - 3′
    3′- TTCATGTCGTA - 5′
    Translate this sequence into protein using the genetic code.
17. Every codon consists of a triplet of base pairs. **Explain** why amino acids cannot be coded with just two base pairs.
18. **Describe** how the structure of mRNA is similar to DNA. **How** does mRNA differ from DNA?
19. DE Cutting a piece of DNA with a restriction enzyme can give DNA fragments with sticky ends or with blunt ends, depending on the restriction enzyme that is used. Write a unified response addressing the following aspects of cutting DNA with a restriction enzyme:
    - **Distinguish** between sticky ends and blunt ends.
    - **Describe** how a DNA fragment with a sticky ends could be produced.
    - **Describe** how a DNA fragment with blunt ends could be produced.
    - **Illustrate** your descriptions with diagrams.
20. The DNA fragment CGTCATCGATCATGCAGCTC contains a restriction enzyme recognition site. **Identify** the site.
21. **Explain** how the presence of an antibiotic-resistance marker gene in a plasmid can be used to determine whether a transformation protocol has been successful.
22. Recently, the Human Genome Project (HGP) was completed. The HGP has provided us with a complete sequence of the human genome. Despite this great advancement, we are far away from realizing the numerous medical treatments that will eventually be made available because of, or as a result of, the project. Scientists are now working on the Human Proteome Project, which involves linking genes to both functional and dysfunctional proteins. **Explain** why there would be limited progress in medical research if scientists were restricted to working only with DNA sequences and not with proteins.
23. DE *Pseudomonas syringae* is a bacterium found in raindrops and most ice crystals. These bacteria act as nuclei for ice crystal formation, catalyzing ice formation at temperatures approaching 0 °C. It does so by producing an ice-nucleation protein in the outer membrane of its cells. Researchers have been able to cleave the gene for this protein from its genome, thereby preventing the bacteria from forming ice crystals. When the genetically engineered "ice-minus" bacteria are sprayed on tomato plants, frost damage is reduced. The presence of the ice-minus bacteria can extend growing seasons, thus increasing crop yields, especially in cold climates. However, environmental groups have raised serious concerns about releasing genetically engineered bacteria into the environment. Write a unified response addressing the following aspects of the use of ice-minus bacteria:
    - **Predict** whether the new microbes could gain a selective advantage over the naturally occurring species?
    - **Describe** what might happen if the genetically engineered microbes mutate?
    - Do you think that genetically engineered microbes should be introduced into the environment? **Justify** your opinion.

Use the following information to answer questions 24 to 26.

Huntington disease is an inherited disorder that manifests itself in abnormal body movements and memory loss that degenerates into dementia and cognitive decline. This disorder is caused by a codon repeat in the Huntington protein gene on chromosome 4. In the normal form of this gene there are fewer than 40 repeats of the codon CAG. More repeats result in the eventual onset of the disease and severity seems to increase with the number of repeats.

24. **Identify** the amino acid specified by the CAG codon.
DE

25. **Explain** what increased inclusions of the CAG codon in the Huntington gene might do to the protein structure of the Huntington protein.
DE

26. **Describe** the steps a lab would take to diagnose the number of CAG repeats on the Huntington gene of an individual. **Identify** the specific technologies and **describe** how they would be used in this analysis.
DE

Use the following information to answer questions 27 and 28.

The first recombinant DNA organisms were bacteria that were altered for commercial purposes to produce a protein product when grown in culture. These recombinant organisms caused little public concern, as they were perceived to be contained within a laboratory or factory. However, subsequent genetic engineering projects have included the release of engineered organisms into the environment. Agricultural transgenic products being grown today include golden rice, insect-resistant maize and cotton, and herbicide-resistant canola and corn, to name a few.

27. **Identify** and **describe** the technologies used to create these recombinant organisms.

28. **Explain** the concerns of those who oppose the use of these organisms, and the benefits touted by their proponents.

Use the following information to answer questions 29 to 32.

A company, Gene Tree, offers kits that can be used to test whether an individual has DNA sequences found most often in the Aboriginal peoples. The tests are compared to known genetic markers in mitochondrial DNA (mtDNA), Y chromosome DNA, and nuclear DNA that are unique to the Aboriginal peoples of North America. Testing to establish the ethnic background of individuals may raise concerns about the use of this information. Historically, there have been political, legal, and moral issues around attempts to identify an individual's ethnicity as distinct from those of others.

29. **Identify** the technique listed above that would best determine paternal inheritance of Aboriginal ancestry. **Explain** your selection.

30. **Sketch** a diagram of meiosis that shows the formation of a human egg. Label the important features and clearly label the ploidy of the key stages. Describe how mtDNA is inherited during the formation of the human zygote, and identify which parent would be contributing the genetic markers for Aboriginal ancestry if they were identified in mtDNA.

31. **Identify** and **describe** two DNA technologies that would be used to carry out these tests.

32. **Identify** two advantages and two disadvantages to both society and individuals that might arise from using DNA technology to trace ethnic patterns of inheritance.

Many of these questions are in the style of the Diploma Exam. You will find guidance for writing Diploma Exams in Appendix A5. Science Directing Words used in Diploma Exams are in bold type. Exam study tips and test-taking suggestions are on the Nelson Web site.

www.science.nelson.com GO

DO NOT WRITE IN THIS TEXTBOOK.

Part 1

1. NR Indicate the correct order, beginning with prophase, of the following events of cell division.
1. Nuclear membrane begins to dissolve.
2. Chromatids move to opposite poles.
3. Chromosomes align along the equatorial plate.
4. Chromosomes reach opposite poles and begin to lengthen.

2. A fertilized mosquito egg has six chromosomes. During mitosis, the egg cell undergoes multiple divisions. Which row shows the correct number of chromosomes found in telophase and interphase?

Row	Number of chromosomes: Telophase	Interphase
A.	3	3
B.	3	6
C.	6	3
D.	6	6

Use the following information to answer questions 3 and 4.

Figure 1 shows four phases of cell division in a plant cell.

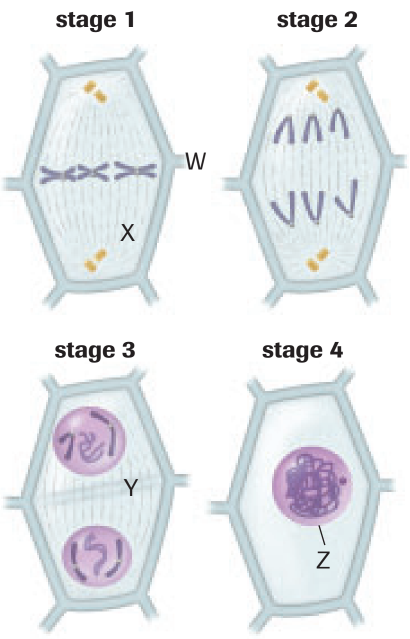


Figure 1

3. NR Identify the phases of cell division in **Figure 1**.

________ prophase   ________ metaphase   ________ anaphase   ________ telophase

4. The correct labels for the structure identified by letters in **Figure 1** are
A. W = centriole, X = centromere, Y = cytoplasm, Z = nucleolus
B. W = centromere, X = spindle fibre, Y = division plate, Z = nuclear membrane
C. W = chromatid, X = centromere, Y = nuclear membrane, Z = nucleolus
D. W = chromosome, X = spindle fibre, Y = chromatin, Z = nuclear membrane

Use the following information to answer questions 5 to 7.

A corn plant with white seeds, a large cob, and small leaves is crossed with a corn plant with yellow seeds, a large cob, and large leaves. All of the F_1 offspring have yellow seeds, large cobs, and large leaves.

5. Identify the row that correctly gives the dominant traits, according to this data.

Row	Seed colour	Cob type	Leaf size
A.	white	large	small
B.	white	small	large
C.	yellow	small	small
D.	yellow	large	large

6. Identify the row that correctly gives the expected traits of the offspring, if a plant from the F_1 generation were cloned.

Row	Seed colour	Cob type	Leaf size
A.	white	large	small
B.	white	small	large
C.	yellow	small	small
D.	yellow	large	large

7. If a plant from the F_1 generation were crossed with a corn plant with white seeds, identify the colour seeds you would expect to see in the F_2 generation.
A. 100 % of individuals would have white seeds
B. 75 % of individuals would have yellow seeds and 25 % white seeds
C. 50 % of individuals would have white seeds and 50 % yellow seeds
D. 100 % of individuals would have yellow seeds

8. Correctly match the cell number in **Figure 2** with the condition. (Record all four digits of your answer.)

______	______	______	______
Turner's syndrome	trisonomic female	will not survive	trisonomic male

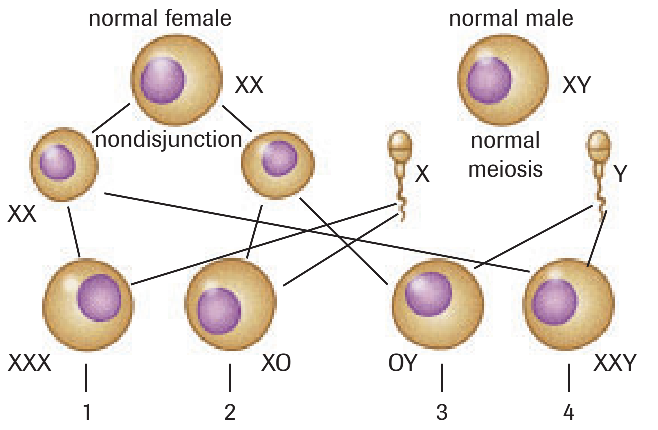


Figure 2

Use the following information to answer questions 9 and 10.

Thalassemia is a serious human genetic disorder that causes severe anemia. People with thalassemia die before sexual maturity. There are over 90 different mutations that can lead to thalassemia. One of the mutations changes the codon TAC to TAA.

9. Identify the row that best describes the type of mutation and its consequence to the structure of the protein.

Row	**Mutation**	**Consequence**
A.	insertion	causes a shift to the reading frame and results in an entirely different amino acid sequence
B.	deletion	causes a shift to the reading frame and results in an entirely different amino acid sequence
C.	substitution	causes a different amino acid at one location
D.	inversion	causes different amino acids for the sequence inverted as reading frame is reversed

10. Select the statement that best describes the codons given in the description of thalassemia.

A. These are mRNA codons as they contain the base uracil.
B. These are mRNA codons as they contain the base thyamine.
C. These are DNA codons as they contain the base uracil.
D. These are DNA codons as they contain the base thymine.

Use the following information to answer questions 11 and 12.

1. Initiation commences when the RNA polymerase binds to the promoter region of the gene to be transcribed.
2. The ribosome continues to move along the mRNA, reading the code in triplets known as codons.
3. When the ribosome moves over, the tRNA containing the growing peptide is shifted over to the P site. A third amino acid, specified by the third codon, is brought into the A site by the next tRNA. A peptide bond is formed between the second and third amino acid.
4. A complementary RNA strand is synthesized in the direction of 5′ to 3′, using one strand of DNA as a template. This step is known as elongation. The complement of adenine in RNA is uracil.
5. New amino acids are added to the chain in the process of elongation, which continues until a stop codon is read in the A site. The stop codons are UAG, UGA, and UAA. At this point, the ribosome stalls.
6. Once the termination sequence is reached by the RNA polymerase, the process ceases. The mRNA is separated from the DNA and the RNA polymerase falls off the DNA molecule.
7. When the start codon is in the P site, a tRNA delivers the amino acid methionine. The tRNA recognizes the codon because of the complementary anticodon.

11. NR Identify the statements that describe the process of transcription. (Record all three digits of your answer in the order in which they would occur in the cell.)

12. NR Identify the statements that describe the process of translation. (Record all four digits of your answer in the order in which they would occur in the cell.)

Use the following information to answer questions 13 to 15.

Genetic inheritance of risk for certain types of breast cancer has long been inferred from its incidence in family clusters. Mutations in either the *BRCA*1 or *BRCA*2 genes accounts for 2 % to 3 % of breast cancers and 9 % of ovarian cancers. People who are identified as having a mutation in either of these genes have a 60 % to 85 % lifetime risk of getting breast cancer and a 15 % to 40 % lifetime risk of getting ovarian cancer. The gene *BRCA*1 is located on chromosome 17 and codes for approximately 1800 amino acids, while the gene *BRCA*2 is located on chromosome 13 and codes for approximately 3400 amino acids.

13. Select the statement that is supported by these data.
   A. Mutations in the *BRCA*1 and *BRCA*2 genes are inherited in an autosomal recessive pattern.
   B. Mutations in the *BRCA*1 and *BRCA*2 genes always cause breast cancer and sometimes cause ovarian cancer.
   C. Mutations in the *BRCA*1 and *BRCA*2 genes cause cancer when influenced by environmental factors.
   D. Mutations in the *BRCA*1 and *BRCA*2 genes always cause ovarian cancer and sometimes cause breast cancer.

14. NR Determine the minimum number of base pairs a *BRCA*2 gene would contain to code for a complete protein. (Record all four digits of your answer.)

15. Identify which of the following statements about *BRCA*1 and *BRCA*2 gene mutations is incorrect:
   A. A woman's risk for genetically linked breast cancer is only elevated if the maternal branch of her family had a history of breast cancer.
   B. Mutations in the *BRCA* genes also increase the risk of ovarian cancer.
   C. Women without mutations in the *BRCA* genes may still be at high risk of getting breast cancer.
   D. A woman's lifetime risk of genetically linked breast cancer is elevated if there is a family history of breast cancer in either branch of her family.

Part 2

16. Genetic testing to identify mutations of the *BRCA*1 and *BRCA*2 genes can be accomplished by gene cloning. **Explain** why a patient might or might not want to have such genetic tests done.

Use the following information to answer questions 17 to 19.

A student observed fertilized eggs of two different species, whitefish and frog, undergoing mitosis. The number of cells in each stage of the cell cycle, at the time the egg masses were prepared and mounted on a slide, were counted. These numbers are presented in **Table 1**.

Table 1 Number of Cells in Specific Stages of the Cell Cycle

Cell cycle stage	Whitefish	Frog
interphase	81	88
prophase	10	6
metaphase	5	5
anaphase	1	0
telophase	3	1

17. DE **Determine** the total time for which cells were in each phase, for both whitefish and frog cells.

18. DE **Identify** the phase of the cell cycle that took the longest time to complete, for both whitefish and frog cells.

19. DE **Sketch** the cell cycle for the fertilized whitefish and frog eggs.

Use the following information to answer questions 20 to 22.

Cancer cells can divide at rates that far exceed those of normal cells. Some drugs used to treat cancer block the action of enzymes that are essential for chromosomal duplication.

20. DE **Why** would these drugs be useful in treating cancer?

21. DE **Predict** the phase of the cell cycle that would likely be affected by these drugs.

22. DE **Predict** the phase of mitosis that might be affected.

23. Approximately 25 plant species make up about 90 % of the human diet. Some scientists have speculated that global warming will reduce plant diversity, making us even more dependent on these species. Our ability to maintain our food supply under these conditions would require advances in genetic engineering, selective breeding, and cloning of plants. **Describe** ways in which these technologies might be used to increase crop production.

Use the following information to answer questions 24 to 27.

Figure 2 is a flowchart showing the stages of meiosis for spermatogenesis.

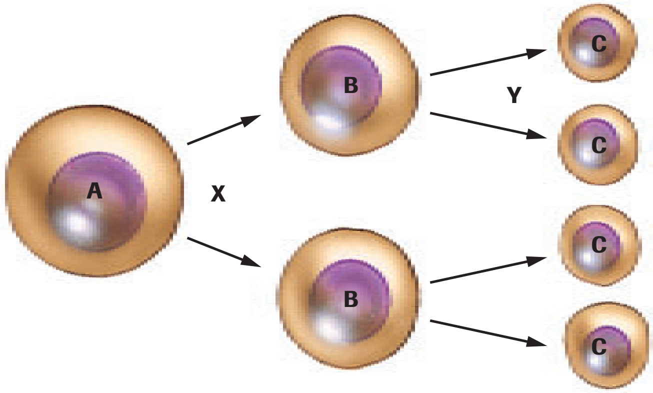


Figure 2

24. **Identify** the stage of meiosis indicated by the labels X and Y on **Figure 2**.
DE

25. **Describe** the events at each stage of meiosis shown on the flowchart in **Figure 2**.
DE

26. **Identify** which cells in **Figure 2** would have haploid chromosomes?
DE

27. Cell A in **Figure 2** contains 44 chromosomes. **Infer** the number of chromosomes that cell C contains.
DE

Use the following information to answer questions 28 to 30.

A normal human sperm cell fertilizes an egg cell containing 24 chromosomes. A lab technician examining a karotype of fetal cells notices trisomy of chromosome pair 21.

28. **Sketch** the karotype.
DE

29. **Predict** how many chromosomes will be found in a muscle cell of the fetus.
DE

30. From the information provided, is it possible to predict the sex of the embryo? **Explain** your answer.
DE

31. Gene therapy is a technique in which defective genes are located and substituted by normally functioning genes. In the future, gene banks may likely be a common source of genes for treating genetic disorders. **List** two potential disadvantages to society of the use of gene banks.

Use the following information to answer questions 32 to 34.

Figure 3 shows the formation of sex cells in a mammal.

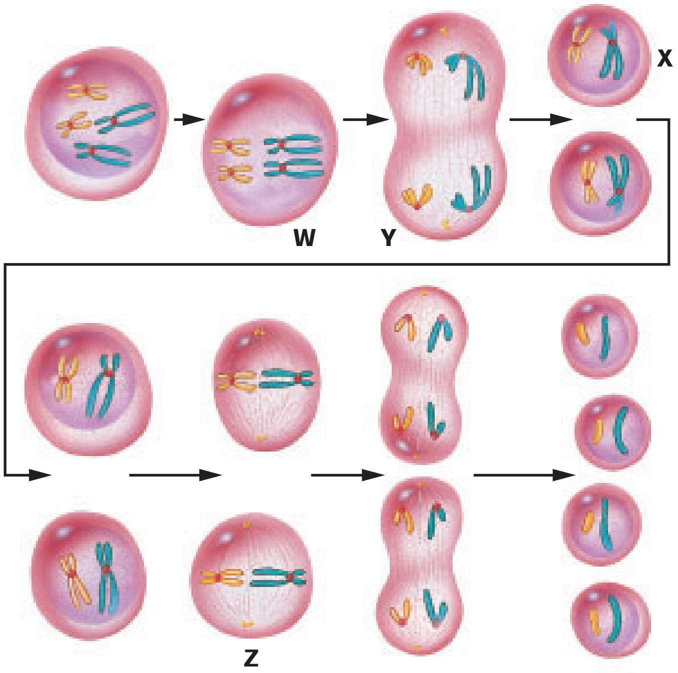


Figure 3

32. Use the diagrams and labels in **Figure 3** to help **explain** the process of crossing over.
DE

33. **Identify** correctly the letter label in **Figure 3** that marks the first haploid cells formed by meiosis. Explain your answer.
DE

34. **Identify** the correct letter label that marks metaphase II. What is happening during this phase?
DE

Use the following information to answer questions 35 and 36.

In guinea pigs, black hair is dominant to white hair, and short hair is dominant to long hair. A guinea pig that is homozygous for both white hair and for short hair is mated with a guinea pig that is homozygous for both black hair and for long hair.

35. **Predict** the phenotype(s) of the F_1 generation.
DE

36. Two members of the F_1 generation are mated. **Determine** the predicted phenotype ratio for the F_2 generation.
DE

37. In chickens, the allele for rose comb (*R*) is dominant over the allele for single comb (*r*), and the allele for feathered legs (*F*) is dominant to the allele for clean legs (*f*). A breeder mates four birds with feather legs and rose combs. The phenotypes of the offspring of these crosses are shown in **Table 2**. **Determine** the genotypes of the parents.

Table 2

Parents	Phenotype of F_1 offspring
rooster A → hen C	all have rose combs; some have feathered legs and some have clean legs
rooster A → hen D	all rose combs and feathered legs
rooster B → hen C	most have rose combs, some have single combs; all have feathered legs
rooster B → hen D	rose and single combs; all have feathered legs

38. In mice, coat colour is determined by more than one gene. For one gene, the allele *C* determines a coloured coat, and the allele *c* determines an albino phenotype. For a second gene, the *B* allele causes activation of a pigment that produces black coat colour. The recessive allele, *b*, causes incomplete activation of the pigment, producing brown coat colour. These two genes are located on separate chromosomes and segregate independently. **Determine** the predicted genotypic and phenotypic ratios of the F_1 generation from the cross *CcBb* × *CcBb*.

39. In your notebook, construct a table to **compare** replication, transcription, and translation. (A comparison includes similarities and differences.) Your table should include the following headings: Process name, Location in cell, Time during cell cycle, Product, Brief summary of process.

40. In actively dividing cells, DNA replication occurs during interphase. **Sketch** the process of replication, using the following segment of DNA as an example:

5′-AAAAATTTAATATATTACAATGGCCCCGCGAT AGTTCGTAGT-3′

3′-TTTTTAAATTATATAATGTTACCGGGGCGCTAT CAAGCATCA-5′

Label and annotate your diagram to describe the process. Clearly indicate the start codon on your diagram.

Use the following information to answer questions 41 to 44.

Tay Sach disease results from a mutation in the gene for the enzyme hexoseaminidase. This mutation is an autosomal recessive disorder. The absence of a correct gene for this enzyme results in an inability to break down fatty material called ganglioside, which causes eventual death as the ganglioside builds up in the brain. There is no effective treatment for this disease.

5′-AUGCAGGUGACCUCAGUG-3′
mRNA sequence for normal protein

5′-AUGCAGGUGACAUACCUCAGUG-3′
mRNA sequence for mutated protein

41. DE Give the amino acid sequence that would result from translation of the mRNA at the ribosome.

42. DE Write the sequence for the normal and mutated protein into your notebook. **Determine** the DNA sequence from which each sequence is transcribed.

43. DE Tay Sach disease is the result of a gene mutation. **Identify** the mutation by circling the changed sequence. Name the type of mutation that has occurred and **explain** the changes that would occur in the protein.

44. DE **Outline** the procedure that you would follow to attempt a gene therapy treatment for Tay Sach disease. Start from the assumption you already know the sequence of the normal gene.

45. **Describe** an advantage and disadvantage to treating individuals with Tay Sachs by applying gene therapy to somatic cells. **Describe** an advantage and disadvantage to treating individuals with Tay Sachs by applying gene therapy to sex cells.

46. The gene for growth hormone has been isolated from human chromosomes and cloned in bacteria. The bacteria produce human growth hormone, which can be harvested in large quantities. The hormone is invaluable to people with dwarfism. Before its development, people with dwarfism relied on costly pituitary extracts. Although the prospect of curing dwarfism has been met with approval, some concerns have been raised about the potentially vast supply of growth hormone. Should individuals who do not have dwarfism but who want to grow a few more centimetres have access to the growth hormone biotechnology? **Justify** your opinion.

47. Review the focusing questions on page 552. Using the knowledge you have gained from this unit, briefly **outline** a response to each of these questions.